MEDICINE AND SURGERY OF TORTOISES AND TURTLES

MEDICINE AND SURGERY OF TORTOISES AND TURTLES

STUART MCARTHUR,
ROGER WILKINSON &
JEAN MEYER

Blackwell
Publishing

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Editorial Offices:
Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ, UK
Tel: +44 (0)1865 776868
Iowa State Press, a Blackwell Publishing Company, 2121 State Avenue, Ames, Iowa 50014–8300, USA
Tel: +1 515 292 0140
Blackwell Publishing Asia Pty Ltd, 550 Swanston Street, Carlton, Victoria 3053, Australia
Tel: +61 (0)3 8359 1011

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First published 2004 by Blackwell Publishing Ltd

Library of Congress Cataloging-in-Publication Data
McArthur, Stuart.
Medicine and surgery of tortoises and turtles / Stuart McArthur, Roger Wilkinson & Jean Meyer.
p.; cm.
Includes bibliographical references and index.
ISBN 1-4051-0889-4
1. Turtles—Diseases. 2. Turtles—Surgery.
[DNLM: 1. Animal Diseases—therapy. 2. Turtles. 3. Veterinary Medicine—methods. SF 997.5.T87 M478m 2003] I. Wilkinson, Roger. II. Meyer, Jean. III. Title.

SF997.5.T87M37 2003
639.3′92—dc21
2002155272

ISBN 1-4051-0889-4

A catalogue record for this title is available from the British Library

Set in 9/12pt Minion
by Graphicraft Limited, Hong Kong
Printed and bound in Denmark
by Narayana Press, Odder, Denmark

The publisher's policy is to use permanent paper from mills that operate a sustainable forestry policy, and which has been manufactured from pulp processed using acid-free and elementary chlorine-free practices. Furthermore, the publisher ensures that the text paper and cover board used have met acceptable environmental accreditation standards.

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DEDICATIONS

I deeply and sincerely thank all those colleagues who have helped with this book. Their names appear throughout the reference section. I am especially grateful to Roger and various members of ARAV (you all know who you are). My parents have played a huge role in converting my 'garbage-spouting' into English. I really have no idea how or why they have sat and managed to read through some of the early drafts of this book. Their motivation probably goes back a long way (I suspect that they are still seeking to try and understand me). Anyway, my heartfelt thanks go to them. This book wouldn't be complete without also giving my thanks, and my love, to Charlotte (my wife) and Ellen and Eve (my children). They have all been very supportive and this book would not have been possible without their help.

A very personal dedication is given to my little sister Donna, and to Michael Vadden MRCVS, as both of them inspired me to become a passionate veterinarian. Sadly both left us whilst this book was being written. Both were dearly loved and they are remembered.

Stuart McArthur

To Sabine with love, and to George and Fin – this is what I did when you woke me up at 2am all those nights.

Roger Wilkinson

I dedicate this book with love to my wife Margit and my son Pitt who share my enthusiasm in nature and who made time and room available for me to work on this book. And to my parents Marie-Therese and Francis who didn't despair during my childhood when piles of terraria and aquaria blocked all the window seats in our home and all kinds of feeds filled our refrigerator and freezer. Their support for my inquisitiveness for all nature-related questions was the most important cornerstone to the origin of this book.

Furthermore I want to thank Stuart and Roger for taking me on board the editorial team and sharing with me the exciting evolution of the book.

Jean Meyer

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FOREWORD

Reptiles are an eclectic group of vertebrates and have long been a source of fascination and interest to the human race. Historically, not all human relationships with different reptiles have been cordial; as biblical and other texts remind us, the reputation of serpents, in particular, was—and in some parts of the world remains—a negative one. Different reptiles have been credited with good or bad fortune and with being portents of a whole range of events. The order Chelonia has escaped much of the bad publicity of the other extant orders of reptiles; indeed many people do not even realise that tortoises and turtles are reptiles. Various chelonian species have tended either to be ignored by human beings, to be utilised for food or as ornaments or to be associated with good luck. The association of tortoises and turtles with longevity in ancient China is one example of the last of these. Turtles have been used as Netsuke figurines, intricately carved sculptures, used as a weight, worn atop the Japanese kimono sash. Obviously such turtles have a special and positive place in Japanese culture. Unfortunately in many other cultures they have become an important source of food or medicinal products. Because of this, many of the worlds chelonians are now in serious decline.

The keeping of chelonians in captivity has long been popular. In many parts of the world tortoises have been kept for companionship or as status symbols. In Darwin's time giant species provided food and ballast for seafarers and this meant that they were often transported to countries far from their origin. Some of these survived the journey and were kept in captivity, often assuming a cultural importance on account of their size or unfamiliarity. This, coupled with the interest over the past century in Western Europe and North America particularly, to keep tortoises as 'pets', has meant that considerable information and opinion has been amassed regarding the care of these animals in captivity.

Although information about chelonians is to be found in the literature and folklore, much of this has tended to be anecdotal and has only infrequently been subjected to scientific review. Data on the basic biology of chelonians, especially anatomy and physiology, have been built up over the years, but until fairly recently this has not been linked with analytical studies on health and disease. We welcome the appearance of this book, which will do much to promote the health, welfare and conservation of chelonians. An attempt is made to bring together as much available information as possible about the biology, management, husbandry, health and disease of captive chelonians. Consequently it will provide much valuable information for both the recent graduate and the more seasoned veterinarian.

As veterinarians who have had a life-long interest in reptiles, we have watched with pleasure and satisfaction a transition from the days when little was known about these animals and how best to tend them in captivity, to the present situation where scientifically based parameters in the literature, and a whole repertoire of diagnostic aids and treatments that can be used, are available. The authors are to be congratulated in presenting the most current information that has been published and providing new techniques that have only recently been adapted to chelonian medicine. Chelonians are some of the most difficult vertebrates to evaluate clinically and the authors bring this out and offer their own approaches for examination and diagnostics.

Tortoises, terrapins and turtles are a fascinating group of animals and a pertinent reminder of the long history of the class Reptilia, and the role that these animals have played in evolution and biodiversity. Now, at the beginning of the twenty-first century, many species of Chelonia face threats. In particular, habitat destruction, illegal harvesting and introduced diseases threaten to eradicate certain species or genera. The illegal trade in chelonians alone is likely to be the cause of the disappearance of some, especially in South East Asia. A greater appreciation of these animals and awareness that they need protection in the wild, and humane care in captivity is vital. This book, with its international orientation (and even a transatlantic Foreword!), will, we believe, contribute much to these ideals.

John E. Cooper, FRCPath, FRCVS
Elliott R. Jacobson, DVM, PhD
April 2003

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LIST OF CONTRIBUTORS

Michelle G. Barrows
CJH Veterinary Surgeons, 15 Temple Sheen Road, London, SW14 7PY, UK

Ian Calvert
Zetland Veterinary Hospital, 32–34 Zetland Road, Bristol, BS6 7AB, UK

John Chitty
Strathmore Veterinary Clinic, London Road, Andover, SP10 2PH, UK

Michaela Gumpenberger
Radiology Clinic, University of Veterinary Medicine, Veterinärplatz 1, A – 1210 Vienna, Austria

Stephen J. Hernandez-Divers
Assistant Professor of Exotic Animal, Wildlife and Zoological Medicine, Department of Small Animal Medicine & Surgery, College of Veterinary Medicine, University of Georgia, 501 DW Brooks Drive, Athens, GA 30602–7390, USA

Charles Innis
VCA Westboro Animal Hospital, 155 Turnpike Road, Westboro, MA 01581, USA

Michael Jessop
Mountain Ash Veterinary Centre, 6 Bruce Street, Mountain Ash, mid-Glamorgan, CF45 3HF, UK

Maud Lafortune
Zoological Medicine Resident, Veterinary Medical Teaching Hospital, College of Veterinary Medicine, University of Florida, USA

Stuart McArthur
Holly House Veterinary Surgery, 468 Street Lane, Leeds, LS17 6HA, UK

Jean Meyer
TierArztPraxis Voelkendorf, Paulapromenade 20, 9500 Villach, Austria

Roger J. Wilkinson
Thornbury Veterinary Group, 515 Bradford Road, Bradford, BD3 7BA, UK

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INTRODUCTION

STUART MCARTHUR, ROGER WILKINSON, MICHELLE BARROWS AND JEAN MEYER

For clinicians who feel that the treatment of domesticated mammals holds no mysteries for them, tortoises and turtles provide a refreshing challenge. There are a variety of reasons for this:

- Chelonians include a diverse array of species. Ernst & Barbour (1989) describe 257 living species of chelonians, which vary widely in their anatomy, physiology, behaviour and environmental requirements. Every year more species are described. It would be a big mistake to assume that two species can be approached in the same way any more than it would be appropriate to treat a sheep and a dolphin alike.
- For many of these species, even basic physiological and ecological data are lacking. We are barely scratching the surface of chelonian pathology. Normal haematological and blood biochemical values have been published for less than 5% of species. The fact that many species are threatened or endangered only complicates the accumulation of such data.
- Chelonians are, on the whole, poikilotherms and are therefore heavily dependent on environmental conditions and husbandry in a way that mammals and birds are not. Behavioural, physical and clinicopathological findings may be completely different at another temperature or when another diet is fed.
- All chelonians have a more or less rigid external shell. This has a really profound effect on our approach to the patient. Coelomic palpation is very limited. Auscultation is severely compromised. Diagnostic procedures such as ultrasonography or biopsy are complicated if the patient chooses to withdraw into the shell, as many do. The clinician is then presented with little more than the external surface of the shell and the feet to examine.

The combination of compromised physical examination and the special significance of environmental conditions mean that the patient's medical history assumes an overwhelmingly disproportionate importance in evaluating chelonian patients. Increased emphasis must also be placed on clinical pathology and diagnostic imaging (particularly ultrasonography and magnetic resonance imaging) although these disciplines are very much in their infancy.

In short, imagine trying to achieve a relatively simple diagnosis, such as pyometra, in a dog by examining only the feet and skin. Where the animal normally spends long periods inactive, fever is not a feature, normal water intake is unknown, normal uterus size is unknown, normal haematological and biochemical findings at this time of year for this sex is unknown, and radiography of soft tissue is not applicable.

DISCLAIMER

Whilst every attempt has been made to ensure the accuracy of the information given, the authors and editors accept no liability for the results of using any data contained in this book. Readers are encouraged to verify all data independently and to use appropriate techniques and licensed drugs wherever they are legally or ethically required to do so.

Keepers of chelonians should be asked to complete appropriate consent forms prior to medical or surgical intervention. Any consent form should inform the keeper of the limitations in reference and therapy data and should include permission to use unlicensed products where the clinician feels appropriate. Clients should always be appropriately informed of the risks involved in undertaking or declining veterinary procedures.

DEALING WITH CHELONIANS

Most chelonian species are covered by Convention on the Trade in Endangered Species (CITES) appendices I or II and are included in the *IUCN Amphibian-Reptile Red Data Book*, published by the World Conservation Union (Groombridge 1982). It may consequently be appropriate to consider referral to a more specialised centre for their treatment, especially when dealing with marine species.

An appropriate treatment vivarium is an essential prerequisite for any practice considering managing a chelonian case. Where referral is not available due to locality or client funds, and where there is sufficient motivation from the practitioner, this book should provide the clinician with appropriate material to manage typical cases. Practitioners are encouraged to communicate with specialist practices for advice and assistance.

INFORMATION REGARDING GENERAL CARE OF CAPTIVE CHELONIANS

The most common reason for presentation of a sick chelonian is incorrect husbandry or nutrition. After stabilising the patient, the clinician is frequently required to provide advice concerning correct husbandry, and may be asked about useful sources of information. This text aims to assist with issues needing consideration in the assessment of general care and to explore and illustrate the fundamental principles involved in management of chelonians in captivity. Without such knowledge it is virtually impossible for a clinician to advise a client as to what needs to be done to improve the health of a sick animal.

A veterinary text such as this cannot claim to summarise overall care for all the chelonians likely to be encountered. The diversity of species, and varied requirements for successful maintenance in captivity, deserve a specialist husbandry text. For up to date information the reader is invited to refer to modern reptile texts, care sheets from chelonian welfare organisations and their internet sites, reptile and zoological veterinary organisations such as the Association of Reptile and Amphibian Veterinarians (ARAV), the British Veterinary Zoological Society (BVZS) and the American Association of Zoo Veterinarians (AAZV); journals

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Fig. 1.1 Veterinarians such as Prof. Elliott Jacobson and Prof. John Cooper have been hugely influential in the development of chelonian medicine and surgery. They are pictured here following the Elliott Jacobson Edward Elkan memorial lecture at the Association of Reptilian and Amphibian Veterinarians (ARAV) 2001.

Fig. 1.2 Annual ARAV meetings, such as this one at Columbus, Ohio, provide an excellent platform for veterinarians to meet and exchange up to date information on reptile care and disease management.

Fig. 1.3 ARAV wet-labs offer veterinarians hands-on experience with specialist tutors. Here Dr Doug Mader gives practical advice on anaesthesia and critical care techniques in reptiles.

such the *Journal of Herpetological Medicine and Surgery (JHMS)* and the *Journal of Zoo and Wildlife Disease (JZWD)*; current peer-reviewed papers (Figs 1.1–1.5).

The majority of species encountered in a veterinary capacity by this author (SM) are Mediterranean *Testudo* species from arid terrestrial habitats. I am greatly indebted to colleagues for providing parallel information on other species, and I have tried to incorporate their experiences with my own.

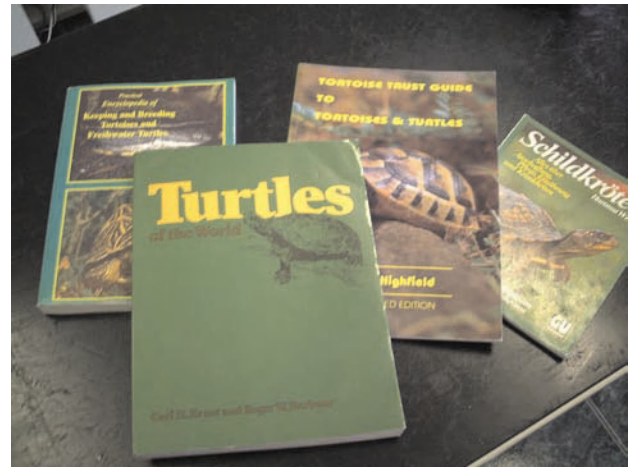

Fig. 1.4 Any practice dealing with chelonians on a regular basis should build up its own library of information. A good place to start is by collecting general husbandry texts, identifying common species likely to be encountered, along with their requirements in captivity. It is also wise to join local husbandry groups such as The British Chelonian Group and The Tortoise Trust (United Kingdom).

Fig. 1.5 Research institutes, zoological parks, nature reserves, conservation parks, museums and libraries have collected a wealth of data on chelonians. The Chelonian Collection at the Natural History Museum, London, is pictured here.

I have given some details from the care sheets provided to all keepers of common chelonian species when attending my own clinic. Providing these allows us to reduce the amount of husbandry advice occupying clinical time with the client, and they are useful aide-memoires for the client.

Little natural history data is available for some poorly-studied species, and the keeper must rely upon the experiences of other keepers or trial and error. The care of such species is best left to experienced keepers. For taxonomy and natural history information, consult a reputable text such as Pritchard (1979), Ernst & Barbour (1989) or Dodd (2001). More recent accounts of individual species may be found in journals such as *Chelonian Conservation and Biology* and the *Journal of Herpetology*.

Caution is advised when dealing with older texts. Our understanding of the requirements of captive reptiles is developing at a dramatic rate, exposing even quite recent texts as being dated and inappropriate. Nutritional advice is available from Ernst & Barbour (1989), Boyer (1992), Innis (1994), Donoghue (1996), Donoghue & Langenberg (1996), Highfield (1996), de Vosjoli

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(1996) and Dodd (2001). In addition, captive-care articles are often published in magazines such as *Reptiles, Reptile and Amphibian Hobbyist* and *Fauna*.

Finally, the publications and Internet web pages of regional, national and international tortoise welfare groups often provide useful husbandry information. Some of the most reputable groups include the British Chelonian Group and Tortoise Trust in the United Kingdom, the Tortoise Trust of the United States, the San Diego Turtle and Tortoise Society, and the New York Turtle and Tortoise Society. For German-speaking readers of the book we recommend the Swiss turtle organisation Schweizer Interessensgemeinschaft für Schildkröten, www.sigs.ch, and the web site of the German Herpetological Society, www.dght.de.

CHELONIAN CONSULTATIONS

Chelonian consultations undertaken in private practice may take longer than most cat or dog consultations. Routine care is generally more complex and detailed questioning of the keeper is usually necessary. When an appointment is being made at a veterinary surgery/office, ensure suitable time is allowed. Information regarding the size, sex and species of the patient should be ascertained. At this author's clinic (SM) we allow half an hour per initial chelonian consultation.

During the initial telephone contact, advice can be given regarding the safe transportation of a chelonian, the form the consultation takes, hospitalisation and diagnostic facilities available at the surgery, and an approximate guide to cost. Details of previous investigations, diagnoses and treatments should always be sought, along with clearance from any previous veterinarian. Clients should be instructed to bring faecal and urine samples if possible. It is obviously helpful for the veterinarian to be provided with as much information as possible about a new case, to facilitate research and preparation. It is unlikely that a clinician can be familiar with the husbandry requirements of all species.

Reptiles of significant size (e.g. more than 25 kg) may be best visited in their normal captive environment. Portable radiography and ultrasound machines are helpful. It is essential that sampling equipment and restraining utensils be brought in anticipation of any potential diagnostic procedures that might be employed.

Welcome packs detailing the care of commonly-presented species can be provided when a client registers with, or arrives at, a clinic. This can give waiting clients something informative to read and prepares them for the questions asked later. Welcome packs can be posted to those reptile owners making telephone contact for care or health advice. However, it must also be remembered that access to this information might influence how clients later describe their chelonian's care.

This author's pack (SM) includes nutrition advice sheets, annual photoperiod sheets, membership details for national and local chelonian welfare groups, species-specific care sheets from these groups, relevant medical insurance application forms, flyers for suitable products, surgery details and road directions if appropriate.

TAXONOMY

As veterinary literature in such specific fields as reptile medicine is often under fire from herpetologists regarding the use of Latin classification systems, we tried to use a recognised source of taxonomic data. The taxonomic list used was published by David (1994). It is clear that the taxons are subject to constant changes and we can't pay respect to all of these as this is not the major goal of this book. In some cases cited literature uses taxons which are not assignable to an English name. These are reproduced as used in the cited text without paying respect to a valid taxonomy.

Ernst & Barbour (1989) described 11 families and 257 species of chelonians and probably another ten or so have been identified since then. All are characterised by possession of a shell consisting of a domed dorsal carapace and a ventral plastron. Two modern 'infraorders', or suborders, of chelonians have arisen: Cryptodira, which demonstrate vertical head retraction, and Pleurodira, which are unable to retract their necks but which lay their heads laterally across the cranial carapacial inlet instead. A taxonomic summary of chelonians is included in the appendices. Some species are identified in Figs 1.6–1.131. Tables 1.1 and 1.2 will also help in identifying species and gender.

Fig. 1.6 Malayan box turtle (*Cuora flavomarginata*): lateral view. (Yellow marginated box turtle.)

Fig. 1.7 Malayan box turtle (*Cuora flavomarginata*): dorsal view. (Yellow marginated box turtle.)

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Fig. 1.8 Malayan box turtle (*Cuora flavomarginata*): ventral view. (Yellow marginated box turtle.)

Fig. 1.9 Juvenile Schweiggers hinged tortoise (also see Figs 1.60–1.63).

Fig. 1.10 Juvenile Schweiggers hinged tortoise (also see Figs 1.60–1.63).

Fig. 1.11 Juvenile Schweiggers hinged tortoise (also see Figs 1.60–1.63).

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Fig. 1.12 Elongated tortoise (*Indotestudo elongata*): anterior view.

Fig. 1.13 Elongated tortoise (*Indotestudo elongata*): dorsal view.

Fig. 1.14 Elongated tortoise (*Indotestudo elongata*): ventral view.

Fig. 1.15 While often listed as a subspecies of *Geoemyda spengleri*, the Japanese leaf turtle (*Geoemyda japonica*) is considered by others to be a unique species.

It is a larger species, with a more domed carapace, and has axillary and sometimes inguinal scutes that *G. spengleri* consistently lacks. *G. japonica* has recently been bred in captivity in the United States.
(Courtesy of C. Innis)

Fig. 1.16 Japanese leaf turtle (*Geoemyda japonica*).
(Courtesy of J. Barzyk)

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Fig. 1.17 Florida box turtle (*Terrapene carolina bauri*): This subspecies of American box turtle is restricted to the Florida peninsula and Keys.

Its radiating shell pattern often leads to its being identified incorrectly as *Terrapene ornata*. *T. c. bauri* can be distinguished by the presence of only three toes on the hind feet, and a uniform yellow or tan plastron. *T. ornata* has a radiating plastral pattern.
(Courtesy of C. Innis)

Fig. 1.18 Asian box turtle (*Cuora amboinensis):* This species is more aquatic than most *Cuora* spp.

It can do well in captivity in a tropical, semi-aquatic to aquatic habitat. It is omnivorous.
(Courtesy of C. Innis)

Fig. 1.19 Yellow-margined box turtle (*Cuora flavomarginata*): These semi-aquatic Asian turtles are very active and outgoing. They may be aggressive to conspecifics, so caution should be exercised when kept in groups.

This specimen is a one month old hatchling. Eggs incubated in moist vermiculite at 28°C–30°C generally hatch in 70–90 days. An opaque, transverse, white band on the egg is an early indicator of fertility (as in many chelonian eggs), and is generally visible within a week of oviposition.
(Courtesy of C. Innis)

Fig. 1.20 Flower-back box turtle (*Cuora galbinifrons galbinifrons*): There are a number of debated subspecies of this Asian species.

They are often very ill and reclusive when obtained through the pet trade, and may require months of daily care before they begin to feed voluntarily. Once healthy, however, they are an active, robust species. *C. galbinifrons* is now listed as critically endangered.
(Courtesy of C. Innis)

Fig. 1.21 Sulawesi forest turtle (*Leucocephalon yuwonoi*): This species was described as a new species from Sulawesi in 1995, and moved to a new monotypic genus in 2000.

It is critically endangered as a result of the Asian food and pet trade, and its geographic isolation. Attempts to protect them within Sulawesi and establish *ex situ* captive breeding groups are underway. While females have brown heads, adult males have striking yellow or white heads.
(Courtesy of C. Innis)

Fig. 1.22 Impressed tortoise (*Manouria impressa*): Rarely has this poorly-known species been kept successfully in captivity.

It is known to come from cool, montane forests in southeast Asia and may feed mainly on mushrooms. Small numbers of juveniles hatched from eggs of deceased imported females are doing well in captivity in the United States.
(Courtesy of C. Innis)

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Fig. 1.23 Keeled box turtle (*Pyxidea mouhotii*): These Asian turtles can be maintained similarly to the American *Terrapene carolina*, but without hibernation.

They are terrestrial omnivores, favouring humid, shaded, forest floor environs in the range of 25°C–30°C. Reluctant feeders can often be tempted by earthworms or strawberries.
(Courtesy of C. Innis)

Fig. 1.24 Spider tortoise (*Pyxis arachnoides brygooi*): This beautiful tortoise from Madagascar was rarely seen in captivity until the late 1990s.

It is a small species, in the range of 10 cm as adults, which is suffering greatly from overcollection for the pet trade.
(Courtesy of C. Innis)

Fig. 1.25 Spider tortoise (*Pyxis arachnoides brygooi*): This hatchling emerged after a seven-month incubation on completely dry sphagnum peat.

Experiences in the United States have shown that a cool diapause is required to stimulate egg development of this species. A successful protocol has been to incubate at 30°C for three months, then 20°C for one month, then 30°C for three months. Embryo vasculature can often be seen by candling within several weeks of the return to warmer temperatures.
(Courtesy of C. Innis)

Fig. 1.26 Spiny hill turtle (*Heosemys spinosa*): These Asian turtles begin life with pronounced flared points around the circumference of their shells. These points fade as the animal grows.

They are terrestrial forest-floor dwellers. Reluctant feeders will often accept banana and can then be converted gradually to a more complete diet.
(Courtesy of C. Innis)

Fig. 1.27 Spiny hill turtle (*Heosemys spinosa*): plastron pattern.
(Courtesy of C. Innis)

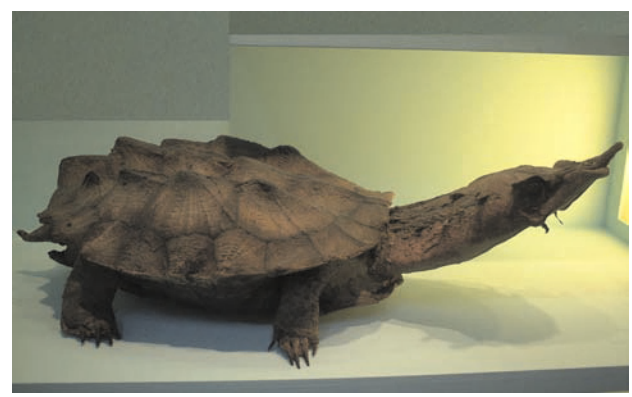

Fig. 1.28 Matamata (*Chelus fimbriata*): The matamata hardly ever leaves water and is carnivorous.

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Fig. 1.29 Red-eared slider (*Trachemys scripta elegans*): view of head and carapace.

Fig. 1.30 Common cooter (*Pseudemys floridana*): view of adult head.

Fig. 1.31 Sawback (false) map turtle (*Graptemys pseudogeographica*): hatchling with small head, pale eyes and notched keel.

Fig. 1.32 Hieroglyphic turtle (river cooter) hatchling (*Pseudemys concinna*).

Fig. 1.33 Ornate box turtle (*Terrapene ornata*): carapace. (Picture courtesy of G. Penney)

Fig. 1.34 Ornate box turtle (*Terrapene ornata*): plastron. (Picture courtesy of G. Penney)

Fig. 1.35 Ornate box turtle (*Terrapene ornata*): side view of head. (Picture courtesy of G. Penney)

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Fig. 1.36 Common or eastern box turtle (*Terrapene carolina carolina*): view of carapace colouration (group of five mature females). (Courtesy of G. Penney)

Fig. 1.37 Common or eastern box turtle (*Terrapene carolina carolina*): view of plastron colouration (group of five mature females). (Courtesy of G. Penney)

Fig. 1.38 Common or eastern box turtle (*Terrapene carolina carolina*): red eyes suggestive of a male. (Courtesy of G. Penney)

Fig. 1.39 Common or eastern box turtle (*Terrapene carolina carolina*): Yellow eyes suggestive of a female. (Courtesy of G. Penney)

Fig. 1.40 Box turtle hybrid, side view.

Fig. 1.41 Box Turtle hybrid, ventral view.

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Fig. 1.42 Hatchling box turtle hybrids showing colour variations within clutch.

Fig. 1.43 Green sea turtle (*Chelonia mydas*): neonate, plastron view.

Fig. 1.44 Green sea turtle (*Chelonia mydas*): neonate, lateral view.

Fig. 1.45 Green sea turtle (*Chelonia mydas*): juvenile, free swimming.

Fig. 1.46 Loggerhead turtle (*Caretta caretta*): neonate, plastron view.

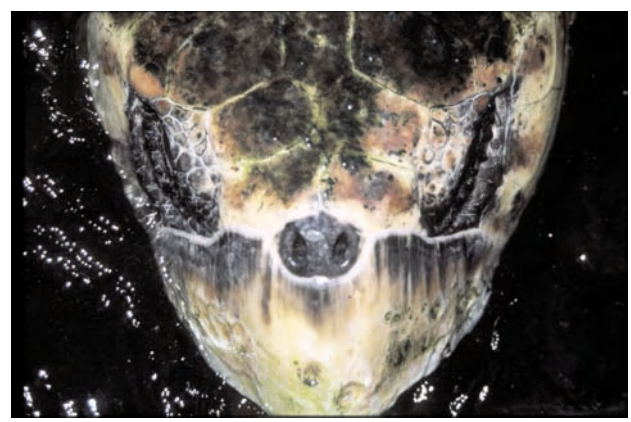

Fig. 1.47 Loggerhead turtle (*Caretta caretta*): adult head.

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Fig. 1.48 Snapping turtle (*Chelydra serpentina*): dorsal view. This aggressive turtle can inflict serious injury.

Fig. 1.49 Snapping turtle (*Chelydra serpentina*): pictured within a suitable aquatic enclosure.

Fig. 1.50 Home's hinged tortoise (*Kinixys homeana*): mature female, anterior view.

Fig. 1.51 Home's hinged tortoise (*Kinixys homeana*): mature female, caudal view showing sharp angulation of the rear portion of the carapace.

Fig. 1.52 Home's hinged tortoise (*Kinixys homeana*): lateral view. The hinge is located between the seventh and eighth marginals and the fourth and fifth costals.

Fig. 1.53 Home's hinged tortoise (*Kinixys homeana*): juvenile, anterolateral view.

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Fig. 1.54 Home's hinged tortoise (*Kinixys homeana*): juvenile lateral view.

Fig. 1.55 Home's hinged tortoise (*Kinixys homeana*): juvenile, caudal view.

Fig. 1.56 Home's hinged tortoise (*Kinixys homeana*): juvenile, plastron.

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Fig. 1.57 Bell's hingeback tortoise (*Kinixys belliana*): lateral view. The caudal carapace slopes more gently than in *Kinixys homeana*.

Fig. 1.58 Bell's hingeback tortoise (*Kinixys belliana*): caudal view.

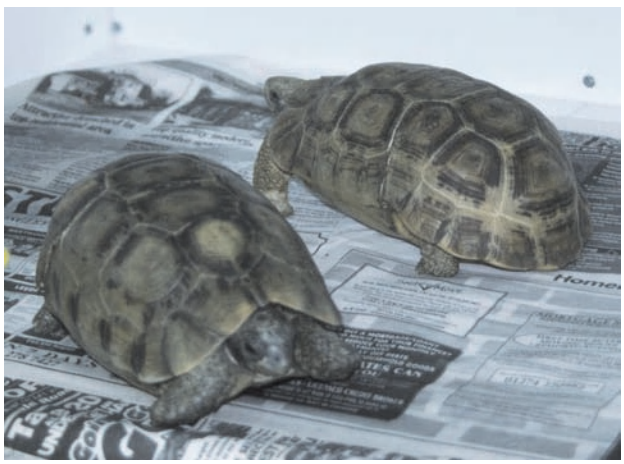

Fig. 1.59 Bell's hingeback tortoise (*Kinixys belliana*): pair of females.

Fig. 1.60 Schweigger's hinged tortoise (*Kinixys erosa*): front view. There are various spurs present around the cranial carapacial opening.

Fig. 1.61 Schweigger's Hinged Tortoise (*Kinixys erosa*): side view. A yellow colouration is often present at the outer edges of the pleural scutes. The scutes are generally a brown colour and may have a central orange colour. The caudal aspect of the carapace does not drop as sharply as *Kinixys homeana.*

Fig. 1.62 Schweigger's hinged tortoise (*Kinixys erosa*): detail of five toes present on both forefeet (the proximal digit has been trimmed).

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Fig. 1.63 Schweigger's hinged tortoise (*Kinixys erosa*): serrated posterior marginal scutes.

Fig. 1.64 African spurred tortoise (*Geochelone sulcata*): two juveniles soon after hatching.

Fig. 1.65 African spurred tortoise (*Geochelone sulcata*): neonate, dorsal view, approximately nine months old.

Fig. 1.66 African spurred tortoise (*Geochelone sulcata*): approximately two years old.

Fig. 1.67 African spurred tortoise (*Geochelone sulcata*): approximately four years old.

Fig. 1.68 African spurred tortoise (*Geochelone sulcata*): approximately fifteen years old.

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Fig. 1.69 African spurred tortoise (*Geochelone sulcata*): head of four year old.

Fig. 1.70 African spurred tortoise (*Geochelone sulcata*): head of two year old.

Fig. 1.71 African spurred tortoise (*Geochelone sulcata*): double spurs of the African spurred tortoise.

Fig. 1.72 Leopard tortoise (*Geochelone pardalis*): anterior view, mature female.

Fig. 1.73 Leopard tortoise (*Geochelone pardalis*): lateral view, mature female.

Fig. 1.74 Leopard tortoise (*Geochelone pardalis*): mature female in the wild (Kenya).

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Fig. 1.75 Double spurs of *Geochelone pardalis*.

Fig. 1.76 Aldabran giant tortoise (*Dipsochelys elephantina*) (David 1994): anterior view showing obvious nuchal scute.

Fig. 1.77 Galapagos giant tortoise (*Chelonoidis nigra* spp.) (David 1994): anterior view. No nuchal scute present.

Fig. 1.78 Californian desert tortoise (*Gopherus agassizii*). (Courtesy of Dr Jim Jarchow)

Fig. 1.79 Californian desert tortoise (*Gopherus agassizii*). (Courtesy of Dr Jay Johnson)

Fig. 1.80 Californian desert tortoise (*Gopherus agassizii*). (Courtesy of Dr Jay Johnson)

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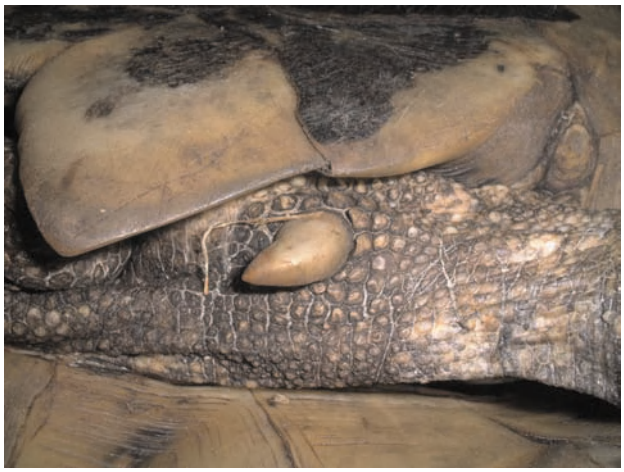

Fig. 1.81 Spur-thighed tortoise (*Testudo graeca*): typical spur of mature tortoise.

Fig. 1.82 Spur-thighed tortoise (*Testudo graeca*): occasionally double or triple spurs are present, with one being obviously dominant.

Fig. 1.83 Turkish spur-thighed tortoise (*Testudo ibera*): anterior view. This tortoise is strong and able to retract deep within its shell, making physical examination of healthy specimens difficult.

Fig. 1.84 *Testudo ibera:* lateral view.

Fig. 1.85 *Testudo graeca* (*Testudo whitei*): carapace.

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Fig. 1.86 Hermann's tortoise (*Testudo hermanni*): ventral view of mature female. A short tail, with tubercle, and a large broad shell indicate female gender.

Fig. 1.87 Hermann's tortoise (*Testudo hermanni*): ventral view of mature male. A long tail, with substantial tubercle, and a moderate, teardrop-shaped shell indicate male gender.

Fig. 1.88 Hermann's tortoise (*Testudo hermanni*): the tail of a mature male can be folded laterally and ends in a substantial spike or tubercle. The tail is capable of inflicting serious trauma to other animals when a sexually active male is housed inappropriately with other animals unable to escape unwanted advances.

Fig. 1.89 Hermann's tortoise (*Testudo hermanni*): head of mature male.

Fig. 1.90 Hermann's tortoise (*Testudo hermanni*):caudal view of mature female showing short tail with tubercle at its tip. In this animal the supracaudal scute is undivided.

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Fig. 1.91 Hermann's tortoise (*Testudo hermanni*): caudal view of mature female showing short tail with tubercle at its tip. In this animal the supracaudal scute is divided.

Fig. 1.92 Horsfield's tortoise (Afghan tortoise, Steppe tortoise, Russian tortoise, four-toed tortoise) *(Testudo horsfieldi)*: juvenile, anterior view. The Afghan tortoise can retreat deep within its tall shell making examination difficult.

Fig. 1.93 Horsfield's tortoise *(Testudo horsfieldi)*: Adult, anterior view. In order to access and examine the head, an assistant can restrain the two forelegs allowing access to the head.

Fig. 1.94 Horsfield's tortoise *(Testudo horsfieldi)*: juvenile, lateral view. The tall bridge and lateral scutes make it easy for this species to defend itself by retreating deep within its shell.

Fig. 1.95 Horsfield's tortoise *(Testudo horsfieldi)*: juvenile, carapace. The shape of the carapace is characteristic. Occasionally a dorsal ridge is present.

Fig. 1.96 Horsfield's tortoise *(Testudo horsfieldi)*: hatchling, dorsal view.

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Fig. 1.97 Horsfield's tortoise (*Testudo horsfieldi*): hatchling, ventral view.

Fig. 1.98 *Testudo ibera:* carapace detail has become obscured as a result of applying oil and polishing the tortoise.

Fig. 1.99 Asian brown tortoise (*Manouria emys*): This large tortoise has several large pointed tubercles on each thigh. Mature adults can reach 60 cm in length.

Fig. 1.100 Asian Brown Tortoise (*Manouria emys*): Eleven marginals line each side of the carapace.

Fig. 1.101 Asian Brown Tortoise (*Manouria emys*): This tortoise is a highland monsoon forest dweller and enjoys soaks and damp environments.

Fig. 1.102 Malayan snail-eating turtle (*Malayemys subtrijuga*): This turtle can reach about 20 cm in length. It lives in slow-moving water and has a distribution from Java, through Malaysia and Thailand, and into Burma.

Its natural diet is snails, worms, aquatic insects, crustaceans and small fish.

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Fig. 1.103 Malayan snail-eating turtle (*Malayemys subtrijuga*) (anterior view): The carapace has a cream-coloured border. The head is black with several cream-coloured stripes.

Fig. 1.104 Malayan snail-eating turtle (*Malayemys subtrijuga*): Moderately-arched, mahogany-coloured oval carapace with three discontinuous keels, the dorsal being the longest.

Fig. 1.105 Orange-headed temple turtle **(***Heosemys (Geoemyda) grandis)*: A large, semi-aquatic Asian turtle that can reach a length of around 45 cm. A well-defined, blunt, median keel is present on the dorsal carapace.

Fig. 1.107 Orange-headed temple turtle (*Heosemys (Geoemyda) grandis*): In the wild these animals are generally considered herbivorous, but captive and temple-confined animals often become significantly omnivorous.

Fig. 1.106 Orange-headed temple turtle (*Heosemys (Geoemyda) grandis*): In Thailand, this turtle is regularly placed in ponds around Buddhist temples (Wats) along with the yellow-headed temple turtle (Figs 1.108–1.113).

Fig. 1.108 Yellow-headed temple turtle (*Hieremys annandalii*): Beak and head.

This large herbivorous turtle may be found in central Thailand, Vietnam and northern Malaysia.

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Fig. 1.109 Yellow-headed temple turtle (*Hieremys annandalei*) (anterior view): This species will eat almost any fruit or green plant.

Fig. 1.110 Yellow-headed temple turtle (*Hieremys annandalei*): Carapace of adult female.

Fig. 1.111 Yellow-headed temple turtle (*Hieremys annandalei*): Plastron of mature female.

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Fig. 1.112 Yellow-headed temple turtle (*Hieremys annandalii*): All toes are heavily webbed.

Fig. 1.113 Yellow-headed temple turtle (*Hieremys annandalii*): In Thailand, Buddhists often place these turtles in ponds around and within temples (Wats), as a method of making merit. Such is the animal illustrated here.

Often the conditions in the ponds are very poor and the animals decline and die, only to be replaced with other, similar animals. Many of the Wat ponds observed by this author (SM) are also overrun with abandoned *Trachemys scripta elegans* which seem to out-compete native Asian species.

Fig. 1.114 Red-foot tortoise (*Geochelone carbonaria*): Front view of head and forelegs. Adult female.

Fig. 1.115 Red-foot tortoise (*Geochelone carbonaria*): Close up of head. Adult female.

Fig. 1.116 Red-foot tortoise (*Geochelone carbonaria*): Adult female, lateral profile.
(Courtesy of Paul Coleman)

Fig. 1.117 Red-foot tortoise (*Geochelone carbonaria*): Adult male, lateral profile
(Courtesy of Paul Coleman)

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Fig. 1.118 Red-foot tortoise (*Geochelone carbonaria*): Carapace of adult female.
(Courtesy of Paul Coleman)

Fig. 1.119 Red-foot tortoise (*Geochelone carbonaria*): Carapace of adult male.
(Courtesy of Paul Coleman)

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Fig. 1.120 Red-foot tortoise (*Geochelone carbonaria*): Plastron of adult female.
(Courtesy of Paul Coleman)

Fig. 1.121 Red-foot tortoise (*Geochelone carbonaria*): Plastron of adult male.
(Courtesy of Paul Coleman)

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Fig. 1.122 Red-foot tortoise (*Geochelone carbonaria*): Anterior profile of hatchling.
(Courtesy of Paul Coleman)

Fig. 1.123 Red-foot tortoise (*Geochelone carbonaria*): Lateral profile of hatchling.
(Courtesy of Paul Coleman)

Fig. 1.124 Yellow-foot tortoise (*Geochelone denticulata*): Adult female, anterior profile.
(Courtesy of Paul Coleman)

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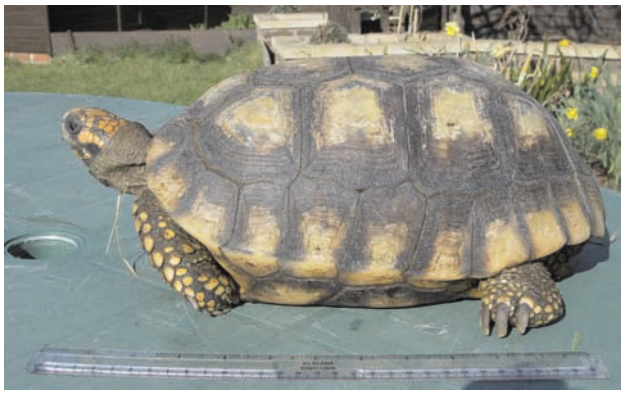

Fig. 1.125 Yellow-foot tortoise (*Geochelone denticulata*): Adult female, side profile.
(Courtesy of Paul Coleman)

Fig. 1.126 Yellow-foot tortoise (*Geochelone denticulata*): Adult male, side profile.
(Courtesy of Paul Coleman)

Fig. 1.127 Yellow-foot tortoise (*Geochelone denticulata*): Carapace of adult female.
(Courtesy of Paul Coleman)

Fig. 1.128 Yellow-foot tortoise (*Geochelone denticulata*): Carapace of adult male.
(Courtesy of Paul Coleman)

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Fig. 1.129 Yellow-foot tortoise (*Geochelone denticulata*): Plastron of adult female.
(Courtesy of Paul Coleman)

Fig. 1.130 Yellow-foot tortoise (*Geochelone denticulata*): Plastron of adult male.
(Courtesy of Paul Coleman)

Fig. 1.131 Yellow-foot tortoise (*Geochelone denticulata*): Anterior profile of year old juvenile.
(Courtesy of Paul Coleman)

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Table 1.1 A limited guide to identification, sexing and dietary habit for some common captive species.

	Identification	Sexing	Diet
Mediterranean tortoises			
Hermann's tortoise *Testudo hermanni*	Horny spike at end of tail, no spurs in thigh region	Males have longer tails, are often smaller than females and have a slightly concave plastron; females are often more oval than the pear-shaped males when viewed from above	Herbivorous—greens, grasses and flowers (80%), vegetables (15%), small quantity of fruit (5%)
Spur-thighed tortoise *Testudo graeca* *Testudo ibera* *Testudo whitei* *Furculachelys nabeulensis*	Spurs/tubercles on medial thighs	Males have longer tails; females often larger and broader than males	Herbivorous—greens, grasses and flowers (80%), vegetables (15%), small quantity of fruit (5%)
Marginated tortoise *Testudo marginata*	Flared posterior marginal scutes	Males have longer tails and are smaller	Herbivorous—greens, grasses and flowers (80%), vegetables (15%), small quantity of fruit (5%)
Horsfield's tortoise *Testudo (Agrionemys) horsfieldi*	Small tortoise with horny tip to tail; tubercles or enlarged scales to sides of tail; four toes on front limbs	Males have longer tails; females may be larger	Herbivorous—greens, grasses and flowers (80%), vegetables (15%), small quantity of fruit (5%)
North American semi-aquatic turtles			
Red-eared turtle/slider *Trachemys scripta elegans*	Distinctive red/orange stripes behind the ears; greenish carapace becoming darker with age	Males smaller, with longer claws on forelimbs once sexually mature; males have longer tails with more distal vent	Omnivorous—dietary preferences change as they age, with young animals being mainly carnivorous and older turtles eating more vegetable matter
North American box turtles			
Three-toed box turtle *Terrapene carolina triunguis*	Hinged plastron; three toes on hind legs; red/orange scales on forelimbs and head	Red iris in males, yellow/brown iris in females; males have longer thicker tails	Omnivorous—greens, vegetables, fruit, mushrooms (30%–50%), worms, snails, millipedes, slugs, pinkies and low-fat dog food (a moderate proportion of the diet)
Ornate box turtle *Terrapene ornata*	Hinged plastron; lighter-coloured, radiating carapacial markings	As above, although iris colour differences not as obvious	Omnivorous—in the wild eat a high proportion of insects, greens, vegetables, fruit, mushrooms (30%–50%), worms, snails, millipedes, slugs, pinkies, and low-fat dog food (a moderate proportion of the diet)
African hingeback tortoises			
Bell's hingeback tortoise *Kinixys belliana* (several subspecies)	Hinged smooth carapace; posterior carapace rounded; darker centres to scutes	Males have longer, thicker tails and a concave plastron; females have flat plastron.	Omnivorous—greens, vegetables, fruit, mushrooms, hay; smaller amounts of worms, snails, millipedes, slugs, pinkies and low-fat dog food (a moderate proportion of the diet)
***Geochelone* species**			
Leopard tortoise *Geochelone pardalis*	Large tortoise; yellow carapace with black markings	Males have longer tails	Herbivorous—high fibre requirement: greens, grasses, hay and flowers (80%), vegetables (15%), small quantity of fruit (5%)
African spurred tortoise *Geochelone sulcata*	Very large tortoise; two to three large spurs either side of tail; elongated and forked gulars	Males larger with longer tails	Herbivorous—high fibre requirement: greens, grasses, hay and flowers (80%), vegetables, (15%), small quantity of fruit (5%)
Red-foot tortoise *Geochelone carbonaria*	Red/yellow scales on head and legs; brown carapace with red to yellow (northern variant) centres to scutes	Males have longer tails, a concave plastron and a narrower 'waist'	Omnivorous—fruits and flowers (60%), vegetables, greens, mushrooms (30%), small quantity of animal protein (10%)
Yellow-foot tortoise *Geochelone denticulata*	Orange/yellow scales on head and legs; light brown carapace with yellow centres to scutes	Males have longer tails and a concave plastron; females are larger than males	Omnivorous—fruits and flowers (60%), vegetables, greens, mushrooms (30%), small quantity of animal protein (10%)
Desert tortoise *Gopherus agassizii*	Large hind feet; narrow head	Males have longer tails; females are smaller than males. Males have obvious mental glands and a large gular scute	Herbivorous—high fibre requirement: greens, grasses, hay and cactus pads (80%), vegetables (15%), small quantity of fruit (5%)
Asian box turtles			
Malayan box turtle *Cuora amboinensis*	Hinged plastron; pointed head with yellow stripes; brown/black carapace	Males have longer tails	Omnivorous—greens, vegetables, fruit, mushrooms and some animal matter; may prefer to feed in water

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Table 1.2 Identifying some Testudo species by tubercles.

No spurs on tail or thighs	• *Testudo kleinmanni* (miniature Egyptian tortoise) • *Testudo marginata* (although occasional spurred specimens have been presented to this author [SM])
Spur on thighs	Tortoises with spurs on both thighs are commonly referred to as *Testudo graeca*, 'spur thighed tortoises', or 'common tortoises'. This group is further divided into several sub-species, the taxonomy of which currently appears to be both confused and controversial. *Testudo graeca (T. graeca graeca)* (North African species from southern Algeria and Morocco, southern Spain and the Balearic Islands) *Testudo ibera (T. graeca ibera)* (southern European species from Greece, Turkey and surrounding regions) *Testudo zarudnyi (T. graeca zarudnyi)* (from the eastern sector of the central plateau of Iran) *Testudo whitei (Furculachelys whitei)* (giant tortoise of Algeria) *Furculachelys nabeulensis* (miniature Tunisian tortoise) The taxonomic identity of spur-thighed tortoises from Libya, Israel, Syria and southwest Turkey presently remains poorly defined.
Spur on tail-tip only	Herman's tortoise (*Testudo hermanni*) is widely distributed over southern Mediterranean Europe, and wild populations can be found in eastern Spain, southern France, Italy, Sicily, Sardinia, the Balkan peninsula, Yugoslavia, Albania, Bulgaria, Romania, Greece and Turkey (Highfield 1996). Two sub-species are recognised: • *Testudo hermanni hermanni* (western population: France, Spain, Italy) • *Testudo hermanni boettgeri* (eastern population: Balkans, Romania, Turkey)
Spur on tail and thighs	• *Testudo (Agrionemys) horsfieldi* (Afghan or Steppe tortoise from Kazakhstan and southern countries of the former Union of Soviet Socialist Republics, Afghanistan, Pakistan, Iran and China)

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INFECTIOUS AGENTS

STUART MCARTHUR

Detailed information regarding infectious agents present in both sick and apparently healthy chelonians is distributed throughout this book. This chapter serves as a simplified summary. The reader is also directed to Chapter 3 and Chapter 7. Because there are many such agents of infection, barrier nursing, described later in the hospitalisation section, is advised during the examination and care of all hospitalised chelonians.

POTENTIAL ZOONOTIC AGENTS

It is important to be aware of the agents carried by chelonians that may affect our own health or that of our staff or families. This author encourages the use of disposable gloves whenever chelonians are professionally examined or handled. Routine disinfection of all surfaces and items in contact with the animal should be undertaken regularly and always between cases. Where wet faecal smears are routinely examined, the possibility of staff contracting salmonellosis from inappropriately stored or disposed samples must be seriously considered. The following information is given for the consideration of clinicians hospitalising and dealing with chelonians. More specific details on these agents are given in other areas of this book.

Salmonella

Salmonellosis is an extremely important reptilian zoonosis (Bolser 1988; Angulo 1997).

Prevalence of chelonian salmonellosis

Because of poor hygiene precautions when handling reptiles, the age group most at risk of salmonellosis is children (Bolser 1988). In the 1970s it was estimated that 4% of American families owned turtles and that 14% of all human salmonellosis cases in the United States (about 100,000 cases per year) were the result of infection from that source (Angulo 1997).

Dessi & Pagli (1992) suggest that 12%–22% of salmonellosis cases in the United States originated in turtles, and describe a convincing case of zoonosis involving a pet turtle, a small child and her mother. Bolser (1988) points out that, hypothetically, pet turtles may become infected with *Salmonella* by their owners. Estimates of the percentage of turtles carrying salmonellosis in the United States have been 12.1%–85% (Johnson-Delaney 1996), with high levels of environmental contamination in breeding ponds on turtle farms being implicated as sources of egg infection and so infection of hatchlings. Savage & Baker (1980) pointed out that the problem was not just restricted to the United States. Human cases involving turtles have also been confirmed in the United Kingdom, Channel Islands, Soviet Union, Germany, Italy, Turkey, Yugoslavia, Canada and Africa (Bolser 1988).

In necropsy surveys of both turtles and tortoises *Salmonella* spp. were identified in turtles and tortoises (Keymer 1978a; Keymer 1978b). In tortoises, *Salmonella newport* was isolated from one Greek tortoise, *S. ebony* from another and *S. arizona* from a third. *S. wandsworth* was potentially associated with colitis in a Bell's hingeback tortoise. In terrapins, *S. arizona* was found in a Gibba turtle (*Phrynops gibba*) and *S. muenchen* was found in a red-eared slider (*Trachemys scripta*) and a Spanish turtle (*Mauremys caspica leprosa*). There are many reports of the isolation of *Salmonella* from hatchling red-eared sliders (McCoy & Seidler 1973; Thorson 1974; Borland 1975; Chiodini & Sundberg 1981). Recent studies of the intestinal flora are described later in this text when discussing digestive physiology, but *Salmonella* isolates were found to be prevalent in studies of the intestinal flora of normal Mediterranean tortoises, *Testudo* spp., and desert tortoises (*Gopherus agassizii*) (Sunderland & Veal 2000; Dickinson *et al.* 2001) and must be considered to be part of the normal flora of terrestrial chelonians.

In general, the isolation of *Salmonella* from chelonians is not associated with disease, but it may be a reflection of hygiene, housing, water quality and diet, with omnivorous species appearing to be greater sources of human infection than herbivorous species. *Salmonella* would appear to be an occasional normal inhabitant of the chelonian digestive tract. Isolates have also been recovered from eggs, ovaries and gall bladder (McCoy & Seidler 1973). Excretion by previously silent carriers during stressful events such as relocation and dehydration has been demonstrated (DuPont *et al.* 1978; Chiodi & Sundburg 1981). There appears to be no obvious way to certify chelonians as being clear of *Salmonella*.

Salmonella screening

Salmonella screening is of dubious value, as results are unreliable and their interpretation would therefore be difficult. When screening is undertaken, it would be best to take repeated faecal samples during a quarantine period and possibly controlled immunosuppression testing (Wright 1997). Faecal culture is far from foolproof and one would expect a high percentage of false negative screens. According to Fox (1974), 38% of a batch of 39 shipments of terrapins, initially certified as being *Salmonella* free, was later found to be excreting *Salmonella*.

Management of Salmonella-positive chelonians

This author would advise all clinicians to barrier nurse all chelonians (screened or not) as though they *were* carrying *Salmonella* and to wear disposable gloves. Disposable gloves are essential when performing faecal flotation and smear examinations. It would seem wise to advise all keepers of chelonians to practice high standards of hygiene in order to reduce the possibility of contracting salmonellosis from their pet.

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Bone (1992) and Johnson-Delaney (1996) suggest that the treatment of salmonellosis in chelonians is ill advised, as it encourages resistance. Attempts to eliminate *Salmonella* spp. from chelonians have been unsuccessful (Johnson-Delaney 1996). It is unclear what percentage of chelonians treated with antibiotics is carrying latent *Salmonella* and may therefore have been exposed to an antibiotic regime without the veterinarian's knowledge. Cooper (1981) suggests there are situations where destruction of reptiles that carry *Salmonella* should be considered.

Prevention of contracting salmonellosis from reptiles

In the United States, the Center for Disease Control (CDC) has published guidelines for the prevention of salmonellosis from reptiles:

(1) Pregnant women, children less than five years of age and persons with impaired immune system function (e.g. AIDS) should not have contact with reptiles.
(2) Because of the risk of becoming infected with *Salmonella* from a reptile, even without direct contact, households with pregnant women, children under five years of age or persons with impaired immune systems should not keep reptiles. Reptiles are not appropriate pets for childcare centres.
(3) All persons should wash their hands with soap immediately after any contact with a reptile or reptile cage.
(4) Reptiles should be kept out of food preparation areas such as kitchens.
(5) Kitchen sinks should not be used to wash food or water bowls, cages or vivaria used for reptiles, or to bath reptiles. Any sink used for these purposes should be disinfected after use (Angulo 1997).

Other zoonotic agents

Table 2.1 lists and gives some details of the other main zoonotic agents of reptiles.

CHELONIAN INFECTIOUS AGENTS

Infectious agents present in chelonians may in some cases infect other hospitalised chelonians, other reptiles or mammals, depending upon their identity. Table 2.2, which is not exhaustive, gives the reader a taste of what we may occasionally be up against.

BACTERIAL AND MYCOTIC AGENTS COMMONLY RESULTING IN OPPORTUNIST INFECTIONS IN CHELONIANS

It is likely that any debilitated, immunocompromised chelonian is liable to become septicaemic as a result of invasion by organisms present within the oral cavity, gut or cloaca. Septicaemia allows infections to disseminate to joints and visceral organs. Many chelonian patients presented to this author are probably immunosuppressed, maintained at inappropriate temperatures and humidity and exposed to poor levels of hygiene. The likelihood of bacteraemia, septicaemia or localised sepsis should be considered in all chelonian patients. Some clinicians feel this justifies routine antimicrobial therapy in virtually all clinical cases presented. Certainly cytological examination of blood smears revealing toxic heterophils and microbes, or a positive bacterial blood culture, would suggest that urgent provision of antimicrobials is necessary. Similarly, radiographic evidence of lytic lesions associated with septic arthritis demands a quick response with antibiotics after bacteriological samples have been harvested.

An extensive list of bacteria isolated from normal and diseased chelonians is provided. Table 2.3 lists some mycotic and bacterial organisms that will give the clinician some idea of potential opportunist pathogens.

Whilst chelonians are far from being the only source, it should be remembered that sick chelonians may act as a reservoir for these types of agents. Inappropriate disinfection and hygiene

Table 2.1 Other potential zoonotic agents of reptiles.

Vibrio/Campylobacter	Harvey & Greenwood (1985) isolated *Campylobacter fetus* from a nine-month-old baby, a two-year-old child and the father of a family suffering from enteric disease. The same organism was isolated from the family's pet turtle. Schmidt & Fletcher (1983) describe the sudden death of a desert tortoise (*G. agassizii*) associated with the presence of *Vibrio cholerae*.
Yersinia	In a study by MacDonald (1998), of the oral flora of chelonians, *Yersinia pseudotuberculosis* was isolated from several chelonians.
Cryptosporidium	Wright (1997) reports asymptomatic *Cryptosporidium* infection of chelonians. Common reptile isolates have not proved infectious to mammalian hosts, and attempts to transmit the human pathogen *C. parvum* to reptiles have also been unsuccessful (Fayer *et al.* 1995; Cranfield & Graczyk 1995). It is probably incorrect to consider reptile-associated *Cryptosporidium* to be zoonotic. However, until further information is available, the possibility of *Cryptosporidium* transfer from reptiles to immunocompromised humans at least should be considered.
Mycobacterium	Whilst *Mycobacterium* infection is relatively uncommon in chelonians, granulomatous infections have been identified by Jacobson (1978), Posthaus *et al.* (1997) and Divers (1998b). It would be advisable to consider euthanasia of animals considered to harbour such agents.
Other agents	*Chlamydophila psittaci*, *Dermatophilus congolensis*, *Borrelia burgdorferi* (tick borne), *Leptospira* spp., *Listeria monocytogenes*, *Flavobacterium meningosepticum*, *Erysipelothrix rhusiopathiae* and *Coxiella burnetti* are described by Blahak (2000).

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Table 2.2 Chelonian infectious agents.

Type of organism	Typical chelonian examples	Transmission details	Effects non chelonian species?	References
Helminths	Oxyurids, (e.g. *Alaeuris, Mehdiella, Tachygonetria, Thaparia* spp.)	Direct life cycle—orofaecal route (Oxyurids, some Ascarids some Trematodes).	Possibly	Satorhelyi & Sreter (1993); Telford (1971); Frank (1981)
	Ascarids (*e.g. Sulcascaris* spp. *Angusticaecum* spp.) Proatractis Flukes (Digenea: *Pronocephalidae*–gastrointestinal; *Hepalotrema* spp., *Learedius* spp.–cardiovascular) Some tapeworms are described	Indirect life cycle—via prey Ascarids Trematodes Cestodes Flukes in marine turtles are thought to have an intermediate host.		Rideout *et al.* (1987); Glazebrook *et al.* (1981)
Viral agents	Iridovirus Herpesvirus Papilloma virus Pox virus Retrovirus Possibly: Adenovirus ∝-flavivirus Lytic-agent-X	Most viral infections are directly transmitted, often with clinically normal carriers and latency. Recrudescence and shedding related to stress. Some, e.g. flavivirus, are indirect and potentially tick borne.	Unknown	Harper *et al.* (1982); Heldstab & Bestetti (1982); Jacobson *et al.* (1982a); Jacobson *et al.* (1985); Cooper *et al.* (1988); Müller *et al.* (1988); Braune *et al.* (1989); Lange *et al.* (1989); Müller *et al.* (1990); Oettle *et al.* (1990); Jacobson *et al.* (1991a); Kabisch & Frost (1994); Pettan-Brewer *et al.* (1996); Westhouse *et al.* (1996); Casey *et al.* (1997); Marschang *et al.* (1997a); Marschang *et al.* (1997b); Muro *et al.* (1998a); Marschang *et al.* (1998a); Marschang *et al.* (1998b); Drury *et al.* (1998); Orós *et al.* (1998); Quackenbush *et al.* (1998); Drury *et al.* (2001)
Amoebae	*Entamoeba invadens*	Direct life cycle. Chelonians generally carry disease asymptomatically. In-contact snakes and lizards may become ill.	Yes	Jacobson *et al.* (1983)
Salmonella	Various	Direct transmission. No chelonian disease. Chelonians are reservoir hosts.	Yes (man)	Keymer (1978a & 1978b); Bolser (1988); Angulo (1997); Pasmans *et al.* (2000)
Vibrio/ Campylobacter	*Campylobacter fetus, Vibrio mimicus Vibrio cholerae*	Direct transmission. In some situations chelonians are potentially reservoir hosts.	Yes (man)	Schmidt & Fletcher (1983); Harvey & Greenwood (1985); Acuna *et al.* (1999)
Coccidia	*Cryptosporidium* spp. *Caryospora* spp. *Eimeria* spp.	Direct transmission. In some situations chelonians are potentially reservoir hosts.	Yes	McAlister & Upton (1989); Wright (1997); Graczyk *et al.* (1998)
Mycobacteria	*Mycobacterium* spp.	Direct transmission.	Probably	Posthaus *et al.* (1997); Jacobson (1978); Divers (1998d)
Hexamita	*Hexamita parva*	Direct transmission.	Probably	Zwart & Truyens (1975)
Mycoplasma	*Mycoplasma agassizii*	Direct transmission.	Unknown	Jacobson *et al.* (1991b); Brown *et al.* (1994)
Chlamydophila	*Chlamydophila* spp.	Direct transmission.	Probably	Homer *et al.* (1994); Vanrompay *et al.* (1994)
Ectoparasites	Ticks Cloacal mites	Direct transmission.	Yes (ticks)	Camin *et al.* (1967); Cooper & Jackson (1981b); Petney & Knight (1988); Frye (1991a); Gould & Georgi (1991)

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Table 2.3 Some opportunist pathogens of chelonians.

Mycotic agents	*Aspergillus* spp. *Geotrichum* spp. *Paecilomyces* spp. *Beauvaria* spp. *Candida* spp.	Direct transmission. In some situations the environment may be the source of the agent.	Jacobson *et al.* (1979); Austwick & Keymer (1981); Tangredi & Evans (1997); Gonzales-Cabo *et al.* (1995); Kostka *et al.* (1997)
Bacterial agents	*Aeromonas* spp. *Mycobacterium* spp. *Pasteurella* spp. *Pseudomonas* spp. *Yersinia enterocolitica*	Direct transmission. In some situations chelonians are potentially reservoir hosts. In some situations the environment may be the source of the agent.	Stroud *et al.* (1973); Fowler (1980); Snipes *et al.* (1980); Lawrence & Needham (1985); Glazebrook & Campbell (1990); Frye (1991a); Jacobson *et al.* (1991b); Posthaus *et al.* (1997); MacDonald (1998)

techniques may result in inadvertent transmission between veterinary patients. Surgical utensils and tables will require extensive disinfection following chelonian use.

When lesions suspected of harbouring acid-fast organisms are removed from chelonians it is prudent to retain a small section of material in refrigeration at the surgery for future mycobacterial culture. Formalin-preserved material will show the presence of organisms but will not be suitable for mycobacterial culture.

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3 ANATOMY AND PHYSIOLOGY

STUART MCARTHUR, JEAN MEYER AND CHARLES INNIS

Anatomy is discussed extensively throughout this book. Further detail is given here in plates as well as in the sections dealing with radiography, ultrasonography, endoscopy, magnetic resonance imaging (MRI) and surgery.

SHELL AND SKELETON

The chelonian appendicular skeleton is quadrupedal and generally has pentadactyl limbs that extend more laterally than those of mammals, although exceptions, such as the three-toed box turtle (*Terrapene carolina triunguis*) and the four-toed or Horsfield's tortoise (*Agrionemys horsfieldi*) exist. Modified limb girdles lie inside the ribs. These are fused to the carapace in Pleurodira but not in Cryptodira. The skull and shell are examples of dermal ossification, although the thoracic and lumbar ribs also join the structure of the shell. There is no sternum in chelonians (Figs 3.1–3.2).

The chelonian beak is similar to the avian beak. An upper keratinised horny beak, known as the *rhamphotheca*, overlies the osseous jaws. The mandibular ramus, where the horny beak attaches, is known as the *dentary*. The beak has no dentition, but is essential in the prehension of food. The chelonian skull is large and devoid of articulations except at the jaw (Bellairs & Kamal 1981). Mandibular muscles are innervated by the trigeminal nerve (Schumacher 1973) and act to close the jaw.

The shell is covered by epidermal tissue, usually in the form of keratinised plates known as *scutes* (Table 3.1), although in some aquatic and semi-aquatic species this is more a leather-like skin (Figs 3.3 & 3.4). New layers of these epidermal plates are added as chelonians grow. However, counting them is an unreliable

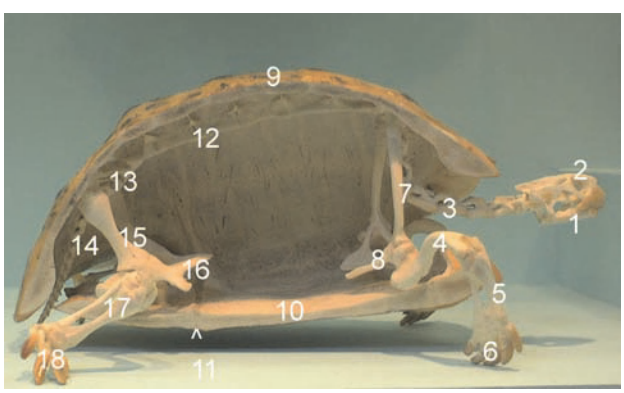

Fig. 3.1 *Geochelone pardalis*, skeletal anatomy.
(1) Mandible
(2) Cranium
(3) Cervical vertebrae
(4) Humerus
(5) Radius and ulna
(6) Carpal bones
(7) Coracoid
(8) Scapula
(9) Carapace
(10) Plastron
(11) Potential plastron hinge
(12) Lumbar vertebrae
(13) Sacral vertebrae
(14) Coccygeal vertebrae
(15) Ilium
(16) Pubis
(17) Tibia and fibula
(18) Tarsal bones

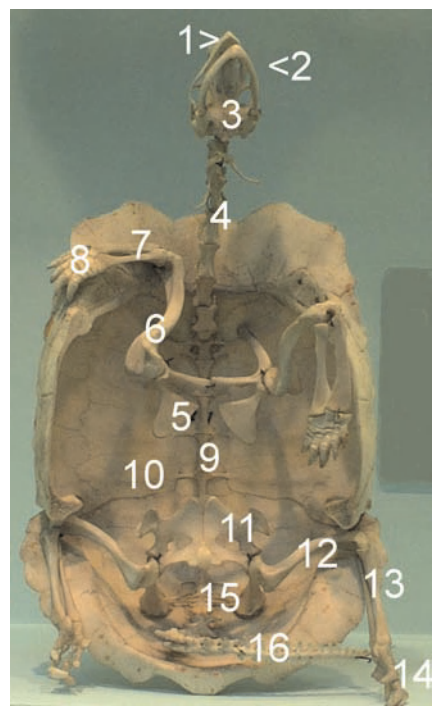

Fig. 3.2 Ventral skeletal anatomy: *Geochelone pardalis*, plastron removed.
(1) Rhamphotheca
(2) Mandible
(3) Cranium
(4) Cervical vertebrae
(5) Scapula
(6) Humeral head
(7) Radius and ulna
(8) Carpal bones
(9) Lumbar vertebrae
(10) Carapace
(11) Pubis
(12) Femur
(13) Tibia and fibula
(14) Tarsal bones
(15) Sacral vertebrae
(16) Coccygeal vertebrae

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Table 3.1 Anatomical terminology.

Term	Definition
Carapace	The upper shell of the tortoise
Plastron	The lower shell of the tortoise
Scute	The horny plates of a tortoise's shell
Vertebral	Central row of scutes along the carapacial spine
Costal/pleural	Scutes between the vertebral and marginal scutes
Marginal	The scutes along the carapace edge (usually 11)
Nuchal	Central carapace scute above the head (marginal)
Gular	Plastral scute below head
Supracaudal	Carapacial scute above tail
Inguinal	Small triangular scute cranial to hind leg
Humeral	Plastral scute behind gular scute
Pectoral	Plastral scute behind humeral scute
Abdominal	Plastral scute behind pectoral scute
Femoral	Plastral scute between anal and abdominal scutes
Anal	Last plastral scute, below tail
Suture	The seam between horny plates
External nares	Paired nasal openings
Beak	Horny tissue covering jaws
Scale	Horny plates of the skin
Tympanic scale	The scale overlying the ear
Cloaca	The chamber into which urogenital and digestive systems empty
Hinge (plastron)	A mobile suture in the plastron (e.g. box turtle)
Hinge (Carapace)	A mobile suture in the caudal carapace (e.g. hingeback)

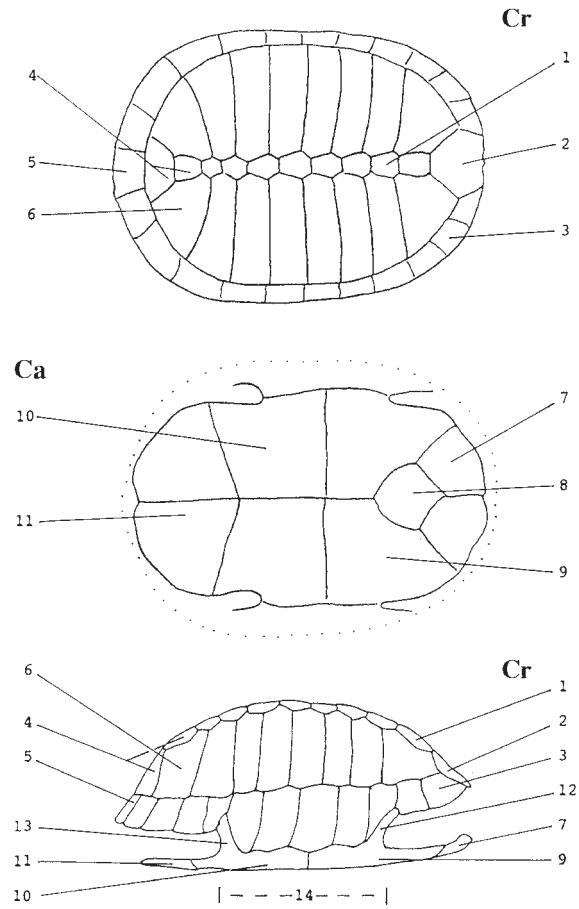

Fig. 3.3 Bony plates of a tortoise.
(1) Neurals
(2) Neckplate
(3) Peripherals
(4) Suprapygals
(5) Pygal
(6) Costals
(7) Epiplastron
(8) Endoplastron
(9) Hyoplastron
(10) Hypoplastron
(11) Xiphiplastron
(12) Cranial Bridge
(13) Caudal Bridge
(14) Bridge
Cr = Cranial
Ca = Caudal
(Courtesy of Jean Meyer)

method of determining age. Dermal-plate growth rates vary, and the number of rings at birth may differ from one animal to another. Epidermal plates are shed regularly throughout life in some semi-aquatic species but seldom in most terrestrial species. This difference has an influence on technique and outcome of plastron osteotomy in these animals. Advice concerning the management of shell fractures and other shell traumas is given later in the clinical sections of this book.

Very young terrestrial tortoises of many species have moderately flexible shells under normal circumstances, but the majority stiffen quickly with juvenile and adolescent calcification. Growth occurs at the scute periphery. Abnormal growth, commonly known as 'pyramiding', appears to relate to high growth rates during the first few years of life. It is occasionally, but not invariably, combined with calcium metabolism abnormalities where the shell is soft and inadequately calcified. Abnormal growth patterns of the scutes can also be a result of inappropriate incubation management (JM: personal observation) (e.g. too high or too low temperatures during shell formation). Physiologically normal soft-shell turtles, *Trionyx* spp., of all ages have reduced ossification and additionally have a leathery skin instead of scutes. Pancake tortoises, *Malacochersus tornieri*, have a flexible shell that allows them to squeeze into rock crevices. *Terrapene* spp., *Pyxidea* spp. and *Cuora* spp. have evolved hinged plastrons, whereas African hingebacks, such as *Kinixys* spp., have a carapacial hinge.

SKIN

The impermeable skin may be thick and scaled as in terrestrial tortoises, or smooth as in some aquatic species. The structure of chelonian skin has evolved to resist the aggressive challenges of the varied habitats in which chelonians are found. These are arid and abrasive for some species (e.g. *Geochelone sulcata*) or wholly aquatic for others (e.g. male *Chelonia mydas*). Chelonian skin is resistant to intense exposure to ultraviolet radiation.

In chelonians, skin structure consists of an outer epidermis, which is derived developmentally from the embryonic ectoderm, resulting in characteristic scales, and a deeper dermis, derived from embryonic mesoderm, which supports, nourishes and gives colour to the epidermis (Davies 1981). The skin has limited

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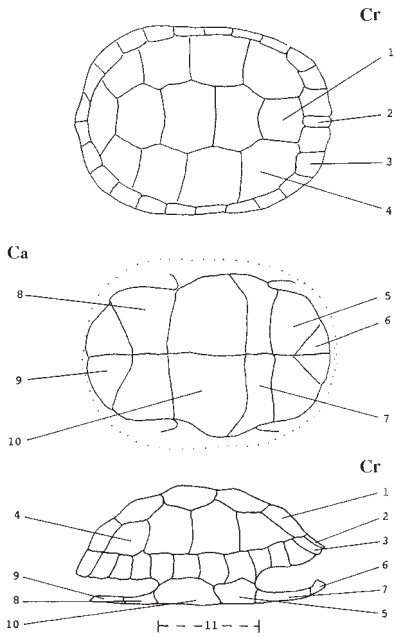

Fig. 3.4 Tortoise scutes.
(1) Vertebrals
(2) Cervicals (nuchal)
(3) Marginals
(4) Pleurals
(5) Pectorals
(6) Gular
(7) Humerals
(8) Femorals
(9) Anals
(10) Abdominals
(11) Bridge
Cr = Cranial
Ca = Caudal
(Courtesy of Jean Meyer)

glandular structures in comparison with most mammals. Shedding does not follow the squamate sloughing cycle, but tends to be uncoordinated and continuous throughout life. Often fragments are shed and sometimes this can be restricted to the softer, more flexible parts of the integument such as the neck and proximal limbs ('T-shirt and shorts') where frictional movements occur most regularly. Further detail on skin structure can be found in texts such as Maderson (1972).

Much of the chelonian shell is an example of a *dermal skeleton*, composed of membranous bone, where there is no preforming with cartilage. In the majority of terrestrial vertebrates dermal bone is retained only in structures such as the cranium and the shoulder blades. Chelonians however retain more general body armour in a manner typical of some ancient fishes (Davies 1981). Species such as the leatherback turtle, *Dermochelys coriacea*, have a leathery protection to the shell as opposed to keratinised scutes.

BODY CAVITIES

Chelonians possess a single *pleuroperitoneal*, or *coelomic*, cavity. This is divided by a horizontal pleuroperitoneal membrane, the *septum horizontale*, which separates the lungs dorsally from the viscera ventrally. There are no true thoracic or abdominal cavities (Figs 3.5–3.6).

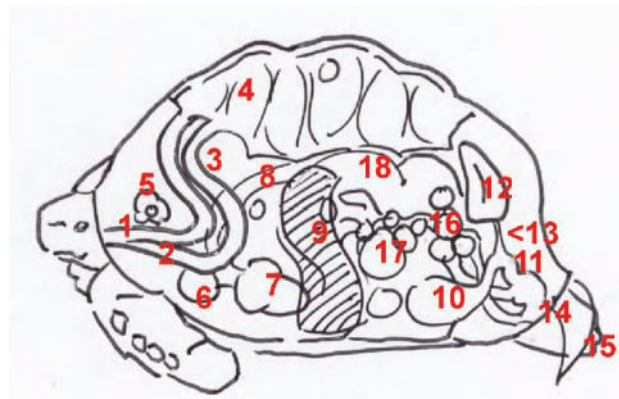

Fig. 3.5 Schematic cross sectional diagram of lateral anatomy of a mature female *Testudo*.
(1) Trachea
(2) Bifurcation of trachea into primary bronchi is cranial in order to facilitate continued respiration when the neck is in flexion
(3) Sigmoid curve of bronchi (when neck is flexed)
(4) Lungs
(5) Thymus (incorporating cranial parathyroid glands)
(6) Thyroid
(7) Heart
(8) Aorta (and adjacent parathyroid glands)
(9) Liver lying centrally within coelomic cavity
(10) Bladder (relatively empty)
(11) Urodeum
(12) Kidney in cross section
(13) Ureter
(14) Coprodeum
(15) Proctodeum
(16) Oviduct (viewed behind immature follicles of left ovary)
(17) Mature follicles of left ovary
(18) Colon descending to coprodeum

Fig. 3.6 Mature female *Testudo kleinmanni* in cross section, immediately after sectioning.
(1) Carapace
(2) Plastron
(3) Head (in cross section)
(4) Neck in flexion
(5) Lung
(6) Spine and epaxial musculature
(7) Stomach (in cross section)
(8) Potential space occupied by bladder within coelomic cavity
(9) Cloaca containing faecal material
(10) Heart (in cross section)
(11) Liver (in cross section)
(12) Area of coelomic cavity occupied by intestines
(13) Septum horizontale (dorsal displacement may be greater here, post mortem, than in life)

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RESPIRATORY SYSTEM

Upper respiratory tract

The upper respiratory tract is entered externally through the external nares, and internally through the oropharynx. From the nares it opens into a keratinised vestibule, which is lined with olfactory epithelium dorsally and mucous epithelium ventrally. This vestibule is divided cranially by a cartilaginous septum into right and left nasal chambers. It is devoid of turbinates and sinuses, and extends caudally into a single passageway lying above the hard palate and opening through a ventral recess into the pharynx at the *choana*. There is no soft palate (Fowler 1980; Jacobson 1997) (Fig. 3.7).

Lower respiratory tract

The lower respiratory tract consists of the glottis, larynx, trachea, paired bronchi and paired, compartmentalised lungs. The trachea, bronchi and lungs are covered by a ciliated glandular epithelium that is poor at eliminating foreign material (Fowler 1980). The trachea divides into paired bronchi relatively cranially in most chelonians and breathing is not impeded when the head and neck are withdrawn (Frye 1991a).

The right and left lungs are similarly-sized, large, sac-like structures with many septae dividing them into peripheral gas-exchanging areas equivalent to mammalian alveoli (Evans 1986).

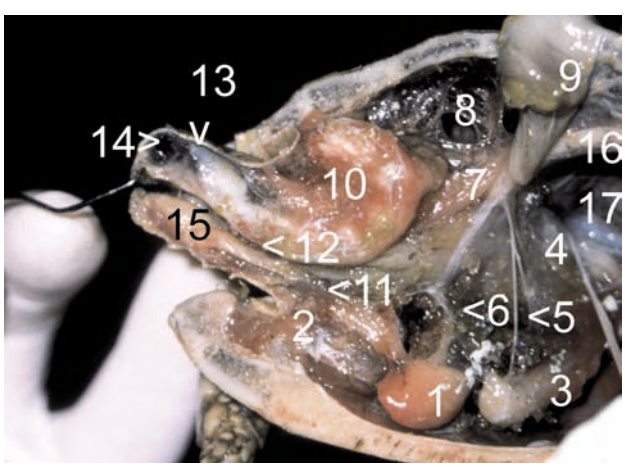

Fig. 3.7 Mature female *Testudo kleinmanni* in cross section, immediately after sectioning. Cranial anatomy.
(1) Heart
(2) Pectoral musculature (epicoelomic injection site)
(3) Small intestine
(4) Mesentery suspending small intestine
(5) Blood vessels supplying section of small intestine
(6) Vessels from heart
(7) Oesophageal remnant
(8) Lung
(9) Stomach (exteriorised)
(10) Neck withdrawn in sigmoid flexion
(11) Trachea (in section)
(12) Pharynx leading into oesophagus (in section)
(13) Brain (in section)
(14) Nasal chamber (in section)
(15) Tongue (in section)
(16) Septum horizontale
(17) Coelomic cavity

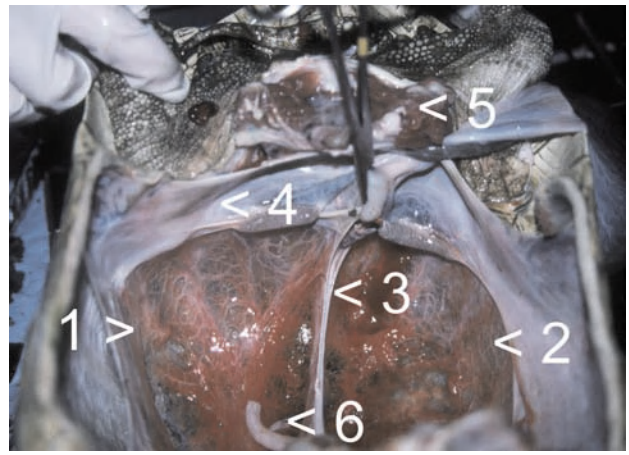

Fig. 3.8 Respiratory anatomy of *Geochelone pardalis:* ventral view, plastron and digestive tract removed and septum horizontale incised and reflected.
(1) Alveolar structure of right lung
(2) Alveolar structure of left lung
(3) Vertical membrane
(4) Septum horizontale, incised and reflected caudally
(5) Pelvic musculature
(6) Right primary bronchus

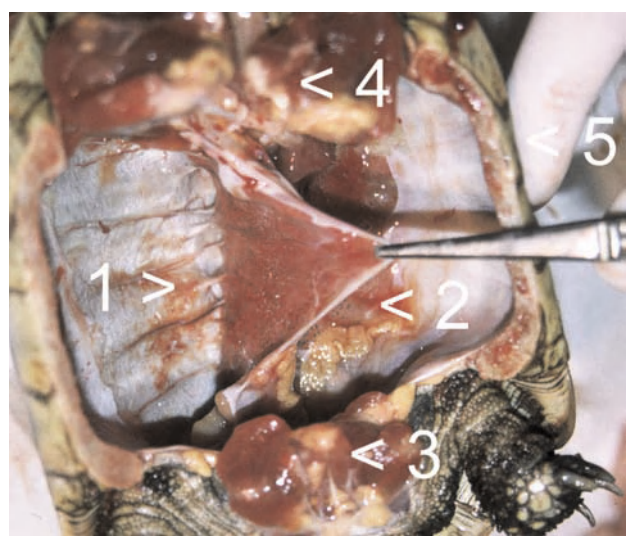

Fig. 3.9 Respiratory anatomy of *Testudo hermanni:* ventral view, plastron and digestive tract removed and septum horizontale incised and reflected.
(1) Costal bones of carapace after overlying lung has been stripped away
(2) Right lung stripped away from carapace
(3) Pelvic girdle
(4) Pectoral girdle
(5) Bridge

Gans & Hughes (1967) suggest that in *Testudo graeca* the right lung may be larger than the left. The lungs lie dorsally against the carapace and above the viscera (Figs 3.8–3.10). Chelonian alveoli differ from mammalian alveoli, in that they are compartmentalised. Oxygen exchange occurs on the reticulated surfaces of these compartments.

The lungs may act as a buoyancy organ in many aquatic and semi-aquatic turtles. Consequently, flotation abnormalities occur in some cases of respiratory disease (Jacobson *et al.* 1979, 1986; Boyer & Boyer 1996; Murray 1996). Flotation abnormalities are

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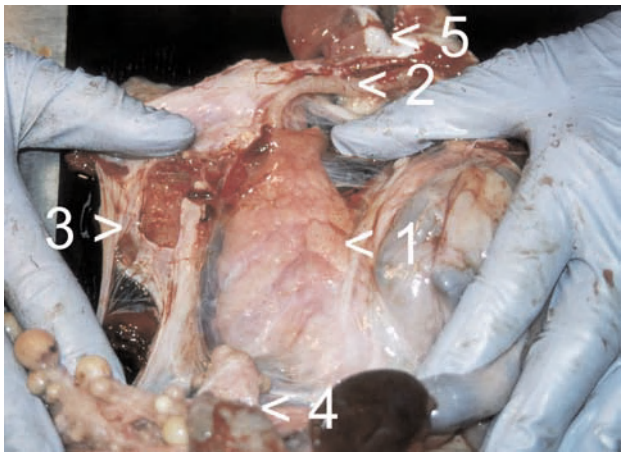

Fig. 3.10 Respiratory anatomy of *Geochelone pardalis: a* single lung and bronchus removed along with associated structures.
(1) Lung tissue (deflated)
(2) Cranial bifurcation of trachea into primary bronchi
(3) Coelomic membrane/pleural membrane
(4) Caudal coelomic viscera such as ovary, oviduct and kidney attached to membranes (3)
(5) Trachea

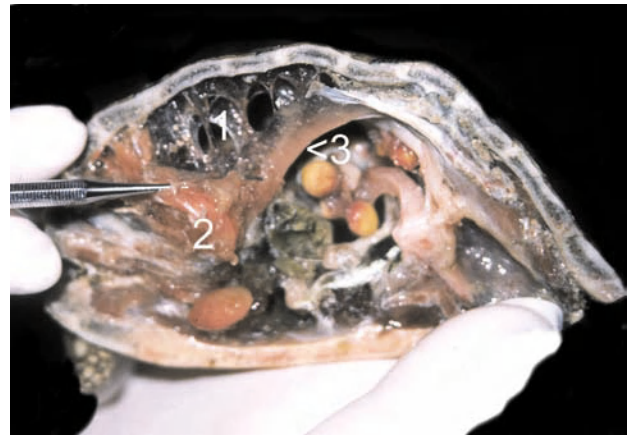

Fig. 3.11 The respiratory tract. The right lung area (1) is displayed by displacing the septum horizontale (3), and the flexed neck (2) ventrally.

also associated with other conditions, such as the escape of air from the lungs into the coelomic cavity or excessive gas in the digestive system, for example, after ingestion of a foreign body such as a plastic bag (Campbell 1996).

Respiratory function

Chelonians do not possess a functional muscular diaphragm separating thoracic and abdominal cavities. The left and right lungs are however distinct, and are separated by a strong vertical membrane. The rigid external carapace prevents ventilation through costal movements. Muscle-induced movements of the viscera, limbs and limb girdles are therefore responsible for alterations in intrapulmonary pressure.

A horizontal pleuroperitoneal membrane (*septum horizontale*), or *pseudodiaphragm*, separates the coelomic cavity from the airspace, but, unlike the mammalian diaphragm, this membrane does not undergo muscular movement to aid ventilation. Diaphragm-derived negative intrathoracic pressure is not required to achieve lung inflation, which is instead mainly controlled by the above-mentioned antagonistic muscle movements of pelvic and pectoral limbs (Gans & Hughes 1967; Wood & Lenfant 1976; Davies 1981). Effectively, the *septum horizontale* is stretched taut and then pulled downwards by limb movements. This increases the area occupied by the lungs, which are tensioned between the carapace and the *septum horizontale*, and facilitates inspiration (Fig. 3.11). Respiration can be maintained despite extensive fractures of the carapace (Boyer & Boyer 1996; SM: personal observation).

Chelonians are very poor at clearing secretions and foreign material from their lower respiratory tract. They are unable to cough, as they lack a muscular diaphragm, and ciliary clearance of respiratory material does not continue all the way up to the glottis (Frye 1991a; Murray 1996). Boyer & Boyer (1996) suggest that this is why pneumonia is often disastrous in chelonians. According to Fowler (1978) and Murray (1996), inflammatory exudates associated with infectious disease tend to accumulate in dependent areas of the lung and this precludes elimination through the bronchi and trachea. Inflammatory exudates of reptiles are caseous rather than liquid as they are in mammals (Jungle & Miller 1992).

Frye (1991a) explains that chelonians have been shown to withstand atmospheres of pure nitrogen for periods of up to eight hours, as they have methods of glycolytic respiration. *Trachemys scripta elegans* has been shown to survive up to 27 hours in a 100% nitrogen environment (Johlin & Moreland 1933). This all helps to explain why they often survive chronic respiratory infections fatal to higher vertebrates that lack the ability to respire anaerobically. Its potential influence upon anaesthesia is also described later.

Respiration of aquatic chelonians

On land, chelonian inspiration is passive and expiration is active, but in water, due to the effect of hydrostatic pressure on visceral volume, the situation is reversed (Jackson 1971; Wood & Lenfant 1976). Wood & Lenfant (1976) describe the diving reflex in *Chelonia mydas* in which breathing following a 20-minute dive is accompanied by a simultaneous increase in heart rate and blood velocity in the pulmonary artery. This maximises the turtle's ability to exchange blood gases.

Many aquatic turtles utilise extrapulmonary respiration during periods of inactivity, but they must surface for air when active (Boyer & Boyer 1976). A *gular pumping* action (movement of water between the mouth, pharynx and choana as a result of movements of the upper digestive tract) is described in some turtles which are able to remain submerged for several hours, but Wood & Lenfant (1976) and Davies (1981) suggest that such gular pumping merely aids olfaction and not respiration. Extrapulmonary respiration in pond, aquatic and sideneck turtles can involve the pharynx, cloacal bursae and, to a lesser extent, the skin (Davies 1981). In the soft-shelled turtle (*Trionyx* spp.), Girgis (1961) suggests that pharyngeal oxygen exchange, through highly-vascularised villiform papillae in the mouth, is responsible for about 30% of underwater oxygen uptake, the remainder occurring across the leathery carapace and plastron. Evans (1986) explains that in warm water these turtles must surface to prevent drowning, presumably due to increased metabolism. According

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to Jackson & Schmidt-Nielson (1996), extrapulmonary oxygen exchange is limited in semi-aquatic species such as *Trachemys scripta*.

Hitzig & Jackson (1978) investigated the central chemical control of normal ventilation of semi-aquatic turtles. These authors found that decreasing the pH of mock cerebrospinal fluid (CSF), used to perfuse the cerebral ventricles of turtles (*Trachemys scripta*) at a variety of temperatures, increased the ventilation rate, even in the presence of a marked alkalosis of arterial blood. Davies & Sexton (1987) produced comparable results in *Chrysemys picta*.

Ishii & Ishii (1986) investigated the chemoreceptors and baroreceptors in the dorsal carotid artery of the tortoise (*Testudo hermanni*). It was shown that an increase in impulse frequency occurred in fine nerve branches of the glossopharyngeal nerve in response to hypoxia, hypercapnia and certain chemicals. The authors concluded that chemoreceptors and baroreceptors exist in the dorsal carotid artery, the aorta and pulmonary artery. These may play a crucial role in the homeostatic management of ventilation and influence vascular resistance.

McLean *et al.* (1989) have investigated pulmonary stretch receptors and their potential influence on respiration and forced ventilation in turtles (*Chrysemys* spp.). Nerve accommodation occurred after prolonged inflation. The authors propose this as a method of stimulating breathing in diving chelonians that breath-hold. West *et al.* (1974) did not reach any clear-cut conclusions as to the factors terminating non-ventilatory periods in *Chelydra serpentina*, where some control must be assumed to be voluntary or behavioural and dependent upon not being underwater.

The dive reflex of chelonians may have special significance during the use of volatile anaesthetic agents where anaesthetic excretion rates may be affected by alterations in respiratory circulation. Diving physiology is described later. Bennett (1998a) suggests many chelonians can perceive a low oxygen environment, become apnoeic and convert to anaerobic metabolism. Such physiological mechanisms as anaerobic respiration and cardiac shunting suit diving and hibernation but complicate inhalation anaesthesia and euthanasia techniques utilising cardioplegic drugs.

Respiratory flora

Non-viral micro-organisms identified in various studies in relation to respiratory pathology are included in Table 3.2. Organisms isolated from normal tortoises are also given. This gives the reader an idea of the type of agents, often gram-negative bacteria, likely to be found in both normal animals and those suffering from respiratory infections.

CIRCULATORY SYSTEM

The three-chambered, valentine-shaped heart lies in the frontal plane, immediately above the plastron, in the midline, cranial to the liver. It has two atria (left and right), and a single, functionally-divided ventricle. The right atrium has a significant muscular wall and receives deoxygenated blood from sources including the left and right precaval veins, the postcaval vein and the left hepatic vein. The blood enters the right atrium via the *sinus venosus*, which also has a thin muscular wall. The left atrium receives blood from the left and right pulmonary veins. Whilst only a single ventricle is present, a series of muscular folds allows control of the flow of oxygenated and deoxygenated blood into the different arterial body circuits. This is described below in the section concerning the *dive reflex*, where the physiological ability to vary the pulmonary circulation is discussed. A significant volume of pericardial fluid may be present even in healthy animals.

The aorta curves dorsocaudally around the heart and two carotid/subclavian arterial trunks run a short distance cranially to divide at the caudal border of the thyroid gland. These are often apparent on ultrasonography. A small thymus lies between the carotid and subclavian arteries on both left and right. A renal portal system is present.

Vascular and lymphatic anatomy is also described by Noble & Noble (1909), Bojanus (1819), Thompson (1932), Ashley (1955) and Ottaviani & Tazzi (1977). Appropriate venepuncture techniques and sites are described in the clinical techniques section later in this book.

Alterations in pulmonary and central circulation (the *dive reflex*)

It is clear that the flow of blood from the heart into the pulmonary vessels is under some form of physiological control. Lutcavage & Lutz (1997) have described the diving physiology of marine turtle species. These authors explore respiratory anatomy, pulmonary gas exchange, oxygen consumption, diving responses, anoxic tolerances and hibernation.

Crossley *et al.* (1998) examined the dive reflex in anaesthetised turtles (*Trachemys scripta*) and found that hypoxia elicited an increase in resistance of pulmonary blood vessels. This effect persisted following administration of atropine and cervical vagotomy, suggesting, but not proving, that the resistance was locally mediated. In this study, acid/base status did not vary. Acid/base status may also affect pulmonary vascular resistance. As division of oxygenated and deoxygenated blood within the chelonian heart is limited, alterations in vascular resistance are likely to have significant influence on the perfusion of organs such as the lungs. Low rates of elimination of inhalation agents primarily excreted by the lungs, such as isoflurane, should therefore be expected in situations where a physiologically-induced increase in pulmonary arterial resistance or dive reflex exists.

It is not clear how exactly physiological signals trigger the reptilian dive reflex. Diethelm & Mader (1999) found that recovery times in anaesthetised iguanas, induced and maintained using isoflurane, were shorter when air as opposed to oxygen was administered, using intermittent positive pressure ventilation (IPPV) throughout the recovery. It would appear likely that circulatory shunting, causing decreased lung perfusion, prevented pneumonic excretion of isoflurane in animals ventilated with oxygen. This suggests that high oxygen tensions also alter pulmonary circulation in these animals. Further work is necessary to clarify the mechanisms affecting excretion of volatile anaesthetics such as isoflurane in reptiles.

Renal portal system

The role of a *renal portal vein* is to maintain blood flow to the tubules within the kidneys at times when glomerular blood flow is

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Table 3.2 Non-viral respiratory organisms.

	Bacteria isolated from normal chelonians	Bacteria isolated from chelonians with respiratory disease		
	Samples are from the oropharynx unless otherwise stated	Samples from the upper respiratory tract (nasal/choanal/ oropharyngeal)	Samples taken from the lower respiratory tract	Samples from related but non-respiratory sites (such as cloaca, mouth or unspecified)
Acinetobacter spp.	MacDonald (1998); Sunderland & Veal (2000): faeces		Evans (1983); Glazebrook & Campbell (1990)	Snipes *et al.* (1980)
Actinobacillus spp.	Sunderland & Veal (2000): faeces			
Aerococcus spp.	Sunderland & Veal (2000): faeces			
Aeromonas hydrophila and other *Aeromonas* spp.	Jacobson *et al.* (1991b); MacDonald (1998); Fowler (1978)	Lawrence & Needham (1985); Jacobson *et al.* (1991b); MacDonald (1998)	Frye (1991a); Jacobson *et al.* (1991b); Glazebrook & Campbell (1990); Stroud *et al.* (1973); Jacobson *et al.* (1991b)	
Alcaligenes faecalis	Lawrence & Needham (1985)	Lawrence & Needham (1985)	Holt *et al.* (1979)	Snipes *et al.* (1980)
Bacillus spp.	Fowler (1978); Lawrence & Needham (1985); Dickinson *et al.* (2001): cloaca	Jacobson *et al.* (1991b)	Jacobson *et al.* (1991b)	Tangredi & Evans (1997)
Bacteroides spp.	Smith (1965): rectum			Stewart (1990)
Bordetella-like spp.	Snipes *et al.* (1980)			Stewart (1990)
Burkholderia cepacia	MacDonald (1998)			
Campylobacter spp.	Harvey & Greenwald (1985); Dickinson *et al.* (2001)			
Cedecea davisae	MacDonald (1998)			
Chlamydophila psittaci			Vanrompay *et al.* (1994)	
Chromobacterium-like spp.	Snipes *et al.* (1980)			
Citrobacter spp.	Snipes *et al.* (1980); Lawrence & Needham (1985); Dickinson *et al.* (2001): cloaca	Lawrence & Needham (1985)		Snipes *et al.* (1980)
Clostridium perfringens				Stewart (1990)
Corynebacterium spp.	Fowler (1978); Snipes *et al.* (1980); MacDonald (1998)	Snipes *et al.* (1980); Jacobson *et al.* (1991b)		Snipes *et al.* (1980)
Edwardsiella tarda			Frye (1991a)	
Enterobacter aerogenes and other spp.	Fowler (1978); Jacobson *et al.* (1991b); MacDonald (1998); Dickinson *et al.* (2001): cloaca	Jacobson *et al.* (1991b)		
Enterococcus spp.	Fowler (1978); Sunderland & Veal (2000): faeces	Snipes *et al.* (1980); Lawrence & Needham (1985)		Snipes *et al.* (1980)
Erwinia spp.	MacDonald (1998)			
Escherichia coli	Fowler (1978); Snipes *et al.* (1980); MacDonald (1998); Dickinson *et al.* (2001): cloaca	MacDonald (1998)	Holt *et al.* (1979); Stroud *et al.* (1973); Jacobson *et al.* (1991b)	Snipes *et al.* (1980); Tangredi & Evans (1997)
Flavobacterium spp.	Lawrence & Needham (1985); Dickinson *et al.* (2001)		Glazebrook & Campbell (1990)	Snipes *et al.* (1980)

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Table 3.2 (*cont'd*)

	Bacteria isolated from normal chelonians	Bacteria isolated from chelonians with respiratory disease		
	Samples are from the oropharynx unless otherwise stated	Samples from the upper respiratory tract (nasal/choanal/ oropharyngeal)	Samples taken from the lower respiratory tract	Samples from related but non-respiratory sites (such as cloaca, mouth or unspecified)
Fusobacterium spp.				Stewart (1990)
Gamella spp.	Lawrence & Needham (1985)			
Klebsiella spp.			Holt *et al.* (1979); Frye (1991a)	
Klebsiella oxytoca	Lawrence & Needham (1985); Jacobson *et al.* (1991b); MacDonald (1998)	Lawrence & Needham (1985); Jacobson *et al.* (1991b); MacDonald (1998)		Snipes *et al.* (1980)
Klebsiella pneumoniae	Fowler (1978); MacDonald (1998); Dickinson *et al.* (2001): cloaca			
Lactobacillus spp.	Dickinson *et al.* (2001): cloaca			
Micrococcus spp.	Fowler (1978); Snipes *et al.* (1980); Lawrence & Needham (1985); MacDonald (1998); Sunderland & Veal (2000): faeces	Snipes *et al.* (1980); Lawrence & Needham (1985)		Snipes *et al.* (1980)
Moraxella spp.	Fowler (1978)	Snipes *et al.* (1980)		
Morganella morganii			Evans (1983)	
Mycobacterium marinum			Posthaus *et al.* (1997a)	
Mycoplasma agassizii		Brown *et al.* (1994); Jacobson *et al.* (1991b); McArthur (unpublished data)		McArthur (unpublished data): eye
Mycoplasma testudinis	Hill (1985): cloaca	McArthur (unpublished data)		
Neisseria animalis		Lawrence & Needham (1985)		
Pasteurella spp. *Pasteurella haemolytica* indole +ve indole –ve *Pasteurella urea*	Fowler (1978); Lawrence & Needham (1985); Sunderland & Veal (2000): faeces; Snipes *et al.* (1980); Dickinson *et al.* (2001): cloaca	Snipes *et al.* (1980)	Frye (1991a); Snipes *et al.* (1980)	Snipes *et al.* (1980)
Pasteurella multocida			Frye (1991a)	
Pasteurella testudinis	Snipes *et al.* (1995); Jacobson *et al.* (1991b); Dickinson *et al.* (2001)	Snipes *et al.* (1980); Jacobson *et al.* (1991b); Dickinson *et al.* (2001)	Frye (1991a); Jacobson *et al.* (1991b)	
Peptostreptococcus spp.				Stewart (1990)
Proteus spp.	Sunderland & Veal (2000): faeces Dickinson *et al.* (2001): cloaca			
Proteus inconstans-like spp.	Fowler (1978)			Snipes *et al.* (1980)
Proteus mirabilis	MacDonald (1998)		Snipes *et al.* (1980)	Snipes *et al.* (1980); Tangredi & Evans (1997)

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Table 3.2 (*cont'd*)

	Bacteria isolated from normal chelonians	Bacteria isolated from chelonians with respiratory disease		
	Samples are from the oropharynx unless otherwise stated	Samples from the upper respiratory tract (nasal/choanal/ oropharyngeal)	Samples taken from the lower respiratory tract	Samples from related but non-respiratory sites (such as cloaca, mouth or unspecified)
Proteus morganii	Lawrence & Needham (1985)	Lawrence & Needham (1985)	Frye (1991a)	
Proteus rettgeri	Lawrence & Needham (1985)		Frye (1991a)	Snipes *et al.* (1980)
Proteus vulgaris				Snipes *et al.* (1980)
Pseudomonas aeruginosa and other Pseudomonas spp.	Fowler (1978); Lawrence & Needham (1985); Jacobson *et al.* (1991b); MacDonald (1998); Sunderland & Veal (2000): faeces; Dickinson *et al.* (2001): cloaca	Lawrence & Needham (1985)	Evans (1983); Frye (1991a); Glazebrook & Campbell (1990)	Snipes *et al.* (1980); Jacobson (1985); Tangredi & Evans (1997); Dickinson *et al.* (2001): cloaca
Rhodococcus spp.	Sunderland & Veal (2000): faeces			
Salmonella spp. (all isolates cloacal/faecal)	McCoy & Seidler (1973); Thorson (1974); Borland (1975); DuPonte *et al.* (1978); Chiodini & Sundberg (1981); Sunderland & Veal (2000); Dickinson *et al.* (2001)			Dickinson *et al.* (2001)
Serratia spp.	Snipes *et al.* (1980); Jacobson *et al.* (1991b); MacDonald (1998)	Lawrence & Needham (1985)	Evans (1983)	
Shigella spp.	Sunderland & Veal (2000): faeces			
Staphylococcus spp.	Fowler (1978); Lawrence & Needham (1985); Snipes *et al.* (1980); Jacobson *et al.* (1991); MacDonald (1998); Sunderland & Veal (2000): faeces; Dickinson *et al.* (2001): cloaca	Snipes *et al.* (1980); Lawrence & Needham (1985); Jacobson *et al.* (1991b)	Frye (1991a)	Lawrence & Needham (1985); Tangredi & Evans (1997)
Streptococcus spp. *Streptococcus faecalis viridans* (haemolytic)	Jacobson *et al.* (1991); Fowler (1978); Sunderland & Veal (2000): faeces; Dickinson *et al.* (2001): cloaca	Jacobson *et al.* (1991); Snipes *et al.* (1980)	Jacobson *et al.* (1991b); Frye (1991a)	Tangredi & Evans (1997); Snipes *et al.* (1980)
Vibrio alginolytica	MacDonald (1998)		Glazebrook & Campbell (1990)	
Xanthomonas maltophila	MacDonald (1998)			
Yersinia enterocolitica	MacDonald (1998); Sunderland & Veal (2000): faeces	MacDonald (1998)		

low. The renal portal vein is a large vessel which arises near the confluence of the *epigastric* and *external iliac* veins and which enters the reptilian kidney centrally. Benson & Forrest (1999) found that in another reptile, the green iguana (*Iguana iguana*), the majority of blood from the hind limbs generally bypassed the kidney, whereas venous flow from the tail entered the renal portal circulation. Blood in the renal portal vein is extensively exposed to the renal tubules and this should therefore result in increased excretion of those drugs that are excreted through tubules when compared with those that are filtrated in the glomeruli.

Holz *et al.* (1994a), Holz *et al.* (1994b) and Holz (1999) investigated the anatomy and function of the renal portal system of chelonians. The portal vein contains valves capable of shunting blood from the caudal half of the body directly into the kidney, or alternatively to the liver and central venous reserve. It is presumed that biochemical factors, including hydration status, affect the degree of portal flow. This may mean that renal levels of drugs injected into the tail (and maybe the caudal limbs) are unpredictable. Frye (1991a) also suggests that bacterial and fungal infections may spread from caudal portions of reptiles to the kidneys through this renal portal system.

Early advice suggested that medications might be better injected into the cranial limbs of a tortoise in order to avoid rapid elimination, or calculations should be made to take this into

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account (Mader 1997; Jenkins 1996; Klingenberg 1996a). This was thought to be most important with nephrotoxic drugs (e.g. aminoglycosides) and drugs that may trigger gout during dehydration (e.g. calcium salt injections used to induce oviposition). However, if a comparable situation to the iguana exists in chelonian species, special consideration should really be given to medications injected intravenously into the dorsal venous sinus or other area of the tail as opposed to the hind limbs.

Holz (1999) has recently concluded that it is unlikely that injection site has any influence over the activity of a drug and that the caudal half of a reptile is available for drug administration. Malley (1999) suggests that adrenaline released during caudal intramuscular injection may beneficially reduce perfusion of the renal portal circulation, which, as Holz (1999) points out, may effectively increase hepatic perfusion. Both Beck *et al.* (1995) and Holz *et al.* (1994) found that no significant difference in drug metabolism was seen when gentamicin (which, like all aminoglycosides, is excreted by glomerular filtration) was injected into the hind rather than the forelimb. For carbenicillin (a significant proportion of which is actively secreted by the tubules) blood levels were slightly lower for the first eight hours in the hind-limb-injected group, but the authors noted that blood levels remained well in excess of the minimum inhibitory concentration (MIC) for relevant pathogens despite the use of a dose half that recommended by Lawrence *et al.* (1986). Holz (1999) concluded that the proportion of blood from the hind limbs directed through the renal portal system is unlikely to be of clinical significance even in dehydrated animals. Whether this is also the case for drugs injected into the dorsal venous sinus of the tail is unclear and, until it has been adequately investigated, it may be best to presume that it is not.

SENSES

Sight

Eye structures include the eyelids, conjunctiva, sclera, cornea, anterior eye chamber, corneal angle, iris, lens, vitreous body, retina, conus papillaris and optic nerve. Comprehensive detail can be obtained from Underwood (1970), Duke (1958) and Walls (1942). The chelonian eyes are located in the orbits of the bony skull. In most chelonians, the eyes are separated medially by bony structures. In Emydinae the interorbital septum is partly, and in the *Trionyx* spp. largely, fibrous (Hoffmann 1890). If an enucleation is attempted, care should be taken not to lacerate the septum and damage the contralateral eye or optic nerve.

Movement of the globe is controlled by superior and inferior oblique, anterior and posterior, and superior and inferior rectus muscles as well as by retractor bulbi and levator bulbi muscles. The origins and insertions of these muscles are described by Underwood (1970).

Eyelids

Two moveable eyelids protect the eye when closed. The lower eyelid lacks a cartilaginous tarsus. The nictitating membrane, which is highly reduced in *Carettochelys*, can be actively withdrawn by the pyramidalis muscle which is innervated by the abducens nerve (Underwood 1970). The inner surface of the eyelids is covered, as in other vertebrates, with conjunctival mucosa.

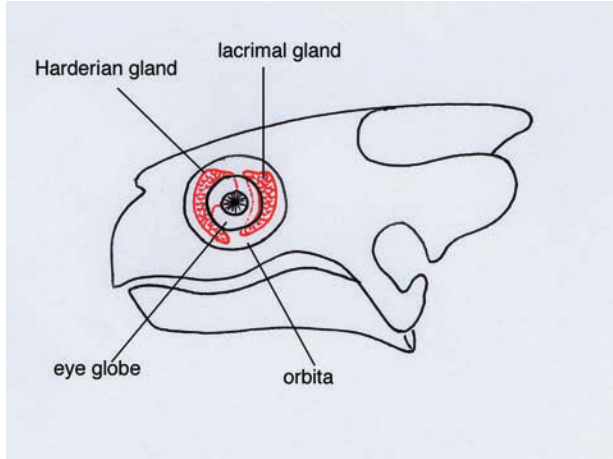

Fig. 3.12 Lacrimal glands: lateral view of a turtle skull showing the positions of the Harderian and lacrimal glands respectively, in relation to the eye globe.
(Courtesy of Jean Meyer)

Glands

Two glands, the *Harderian* and *lacrimal* glands (Fig. 3.12) open into the ventral conjunctival sacs through several separate ducts. The ducts of the Harderian glands drain into the craniomedial angle of the eye between the *membrana nictitans* and the conjunctiva bulbi. The Harderian gland itself lies in the nasal area and the lacrimal gland in the temporal part of the orbit, respectively in the cranio- and caudomedial parts of the eye. They are excavated in a way to fit the globe snugly. Marine turtle lacrimal glands, as well as those of species living in brackish water, aid in the excretion of salts.

The secretions of the glands are purely aqueous. Because there are no nasolacrimal ducts, tears are lost by overflow, evaporation or absorption by the conjunctival mucosa (Millichamp *et al.* 1983). Evaluation of tear production is subjective as no normal values for Schirmer's tear test are available for chelonians.

In hypovitaminosis A, the glands undergo severe histopathological changes. Epithelial proliferation and desquamation lead to the building up of large cysts. The cysts are sterile but lead to enlargement of the eyelids, as the cysts never burst and the whole glandular mass is retained within the lids (Elkan & Zwart 1967). Eventually, the animal becomes unable to open his eyes, and this seriously interferes with the prehension of food. If disease continues to progress, the outer layers of the cornea become cornified and large amounts of dead epithelium become entrapped within the precorneal and conjunctival space.

Sclera

The sclera is composed of fibrous and cartilaginous tissue and contains in its anterior part a variable number of ossicles. These ossicles form a ring that supports the globe with its generally convex shape. Underwood (1970) gives detailed information on the numbers, shape and arrangement of these ossicles in the different chelonian species.

Cornea

The cornea of chelonians lacks a Bowman's layer. The epithelium is very thick and is lined on the inside by a thin Descemet's

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membrane. The shape of the cornea is variable and the subtended angle varies between 70° in *Emys* and 38° in *Caretta* (Underwood 1970).

Anterior chamber

The shape of the anterior chamber is maintained by aqueous humor production, which is drained as in other vertebrates through the canal of Schlemm at the corneal periphery. The iridocorneal angle has similarities to that of mammals, although it is less well developed. The balance between production and draining of the aqueous humor is responsible for the intraocular pressure (IOP). Selmi *et al.* (2002) investigated the IOP of the red-footed tortoise (*Geochelone carbonaria*) by means of electronic applanation tonometry. The mean IOP values for the right and left eyes were 14.5 +/– 8.2 and 15.7 +/– 9.3 mm Hg, respectively. They couldn't show any decrease of IOP with age, as is the case in alligators, and conclude that this may not be so in chelonians.

Iris

The iris has a well-developed sphincter. Its aperture is under voluntary control and can't be influenced by mydriatics. If the posterior parts of the eye are to be investigated, mydriasis can be achieved either by general anaesthesia (especially with ketamine) or by the use of topical muscle relaxants. Periods of mydriasis achieved vary from a few minutes to several days in crocodiles (Millichamp *et al.* 1983). In birds, topical application of vecuronium bromide (4 mg/ml), 2 drops every 15 minutes for 3 instillations, gives reliable results. Maximum mydriasis occurs within one hour and lasts in European kestrels for four hours. No comparable data are available for chelonians but the anatomy and physiology of the chelonian eye make comparable results probable. Secondary effects of these drugs may be flaccid paralysis of eyelids and neck or more generalised paralysis if too much drug is used. Curare (tubocurarine 2%, 0.1 ml) has been used in adult crocodilians to achieve mydriasis by instillation into the cranial eye chamber (Millichamp *et al.* 1983). The vascular supply of the iris and the anatomy of the ciliary body are described in detail by Underwood (1970).

Lens

The lens is suspended by zonula fibres arising from the ciliary body and inserting at the capsule of the lens. The lens is strongly curved in *Caretta* spp. as an adaptation to their predominantly aquatic way of life. In terrestrial *Testudo* species the lens is flatter and the power of accommodation is less.

Retina

The retina is avascular. Nutrition is provided through choroidal vessels and a vascular projection from the optic nerve head, the *conus papillaris*. This is the reptilian equivalent of the avian *pecten* and projects into the vitreous. The fundus can be investigated under mydriasis by use of an indirect ophthalmoscope and a 20 D lens. As refraction is variable in different species, depending on their ecology (aquatic or terrestrial), different diopter lenses may also be tried.

The chelonian retina contains more cones than rods, enabling discrimination between colours. Some vision may be outside of the visible spectrum as many animals behave very differently where significant ultraviolet radiation is present (SM: personal observation).

Colour preference seems well established in chelonians. Many species of North American box turtles (*Terrapene* spp.) are attracted to red and orange (Dodd 2001) and many *Testudo* species are attracted to red and/or yellow (SM: personal observation). These traits have been utilised by many manufacturers of commercial foods. Herbivorous species seem to recognise green foods without fail.

Some degree of low light intensity or night vision is present in many terrestrial species. Species with large globes in relation to overall head size, such as hingeback tortoises (*Kinixys* spp.), red-footed tortoises (*Geochelone carbonaria*) and many box turtles (*Terrapene* spp.) seem better adapted to seeing at low light intensities. This adaptation is likely to have evolved as a result of the light levels present in their natural habitat.

Ophthalmic abnormalities

Conditions of the chelonian eye are described elsewhere throughout this book. Any animal that is inactive, poorly-mobile and failing to feed appropriately should have an ocular examination and a check of visual reflexes (as described later). Sight is very important to most chelonians and its loss has a profound effect upon behaviour. All the blood tests in the world will not indicate that a tortoise is blind. Sometimes a clinician will need to observe the patient for a while and then examine the eyes in order to come to a diagnosis.

Eyes are often damaged in hibernating species when animals are exposed to subzero temperatures. Degree of sight impairment, recovery and signs such as intraocular haemorrhage vary between cases. Eye infections also result from contamination of ocular structures by foreign material. Conjunctival hyperplasia associated with hypovitaminosis A has been recorded frequently in animals such as *Trachemys scripta*. Viral infections may affect the eye and periocular tissues. This author (SM) has encountered a transient dry eye associated with herpesvirus infection, although this is more commonly associated with conjunctivitis. Some animals with profound metabolic diseases, such as chronic follicular stasis and associated hepatic lipidosis, appear to have greatly reduced visual reflexes, which may improve or return to normal following successful therapy. In such cases some form of encephalopathy may impair sight.

Olfaction

Chelonians have a well-developed olfactory system, with extensive chemosensory cells within the nasal epithelium. In many aquatic animals, gular movements, where water is pumped through the nasal chamber by pharyngeal contractions, are thought to be olfactory rather than respiratory. Taste buds are present throughout the oral epithelium, but little is known about the importance of taste in chelonians and how it may be altered by local and systemic disease. Smell and taste do however appear to be important in relation to appetite and normal feeding and many animals can be seen to nose food repeatedly in a smelling action before attempting to eat it.

Many animals seem able to distinguish between foods with and without medications (such as enrofloxacin) in them. Some appear to show distress at the taste of some oral medications and

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claw at their mouths following their administration. Many animals will show a preference for foods which have been sweetened by the addition of dilute sugar solutions. This is a preference utilised by some manufacturers of commercial reptile vitamin sprays. Other animals will preferentially eat fruits that have begun to rot, although this preference may also involve olfaction. Sight-impaired animals often respond positively to the smell of food when it is rubbed between the clinician's fingers immediately before their nares. Allard (1949) and Fitch (1965) both found that box turtles were able to discriminate between similarly-wrapped food and non-food items using their sense of smell.

Hearing

Chelonians have no external ears. The visible part of the chelonian ear is formed by a layer of simple undifferentiated skin which forms the tympanic membrane. This lies at each side of the head, well behind the eye, at the level of the corner of the mouth. The skin of the tympanic area is thinned in the middle and care should be taken not to damage this structure during restraint of the head. Tympanic membranes protect the middle ear cavities, which are connected to the pharynx via the eustachian tubes.

The middle ear cavity is separated by a large process of the quadrate bone into a lateral tympanic cavity and a medial *recessus cavi tympani* (Fig. 3.13). In cases of infections of the middle ear, pus is generally located in the lateral part of this chamber. The inner ear is protected by this bony process.

The sound-receptive part of the ear consists of the tympanic membrane, the underlying *extracolumella*, a cartilaginous disc which is connected to an ossicular process, and the ossicular process, the *columella*. The columella consists of a thin osseus shaft penetrating through a small hole in the bony ventral process of the quadrate bone to join the inner ear where it ends in a funnel-shaped plate. Exact anatomical details for different species are given by Wever (1978) and Baird (1970).

As the tympanic membrane moves, a rotational movement is transferred to the inner ear via the extracolumella and columella.

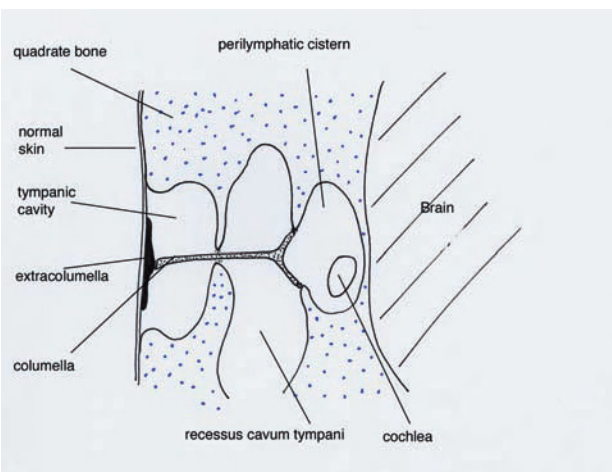

Fig. 3.13 Ear: dorsal view. Schematic view of the ear of a turtle seen from above in a frontal section. The anatomy of the ear differs from species to species. Details on the ear are given in the text.
(Courtesy of Jean Meyer)

The ears may be more important for balance than for hearing, as chelonians are capable of hearing only low tones, many of which may be associated with ground vibration as opposed to being airborne (Wever 1978). The maximum sensitivity in Cryptodira is usually in the region of 100 to 700 Hz and in Pleurodira perhaps an octave higher (Wever 1978).

According to Dodd (2001), box turtles suffering from middle ear infections hear at 20–40 decibels less than healthy animals. In many species, surgery to ear abscesses, where the contents of the middle ear are scooped out and the tympanic membrane is resected, does not seem to affect the future behaviour or balance of the animal unduly. Wever & Vernon (1956) showed that opening of the tympanic cavity and removing part of the lateral wall had no effect on response to low tones and produced only slight variations for high tones.

The small-sized tympanic cavity appears to produce no significant resonance. If, however, the conductive mechanism of the columella is transected, an important decrease in sound perception is measurable (Wever 1978). It is important, therefore, to preserve the columella during removal of an aural abscess. Vigorous cleaning with a curette or similar devices should not be attempted.

GASTROINTESTINAL SYSTEM

Figures 3.14–3.19 below provide diagrammatic and photographic representations of the chelonian digestive system.

Upper digestive tract

The upper digestive tract consists of the beak and jaw, buccal cavity (including the tongue, oropharynx and choana), the pharynx and the oesophagus. The pharynx leads into the oesophagus, which runs down the left side of the neck and may assist in the mechanical breakdown or digestion of food in some species (Skoczylas 1978). Marine turtles have a large oesophageal papilla that allows ingested food items to be retained and ingested while unwanted sea water is rejected back through the oesophagus and out of the nose or mouth. The superficial layer of the oesophageal mucosa contains glandular ciliated epithelium, which can transport small particles in the direction of the stomach (Fox & Musacchia 1959; Guibé 1970; Luppa 1977). Oral examination is described later.

Lower digestive tract

The lower digestive tract consists of the stomach, small and large intestines and cloaca. The pancreas and liver also play an important role in digestion (Fig. 3.20).

Stomach

The stomach is simple and fusiform, running across the caudal face of the liver with the fundus on the left and the pylorus central or slightly to the right. In *Testudo* spp. the cardia is characterised by thick, pillow-like folds, which act as a sphincter (Parsons & Cameron 1977). According to Luppa (1977) there is no thickening of the *tunica muscularis* in the pyloric region. However, Meyer (1996) found a distinct muscular pyloric sphincter in histological preparations of *Testudo hermanni*.

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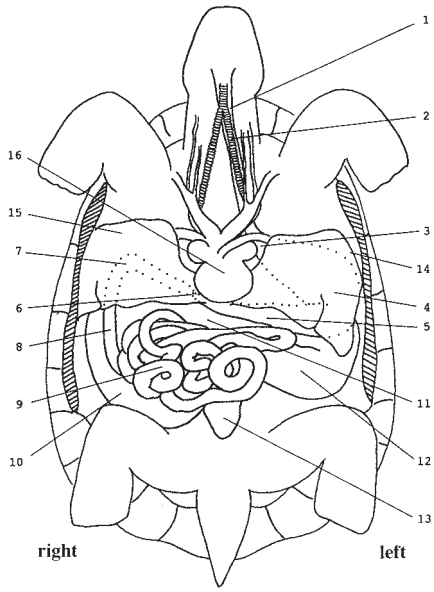

Fig. 3.14 Digestive tract of a female Hermann's tortoise (*Testudo hermanni*). Plastron, bladder and reproductive tract are removed. Ventral view, schematic.
(1) Oesophagus
(2) Left bronchus
(3) Cardia
(4) Stomach, covered by the liver
(5) Pars pylorica of the stomach
(6) Pylorus
(7) Cranial duodenal flexure, covered by the liver
(8) Descending duodenum
(9) Small intestinal loops
(10) Ascending colon
(11) Transverse colon
(12) Descending colon
(13) Rectum
(14) Left liver lobe
(15) Right liver lobe
(16) Heart with major vessels
(Courtesy of Jean Meyer)

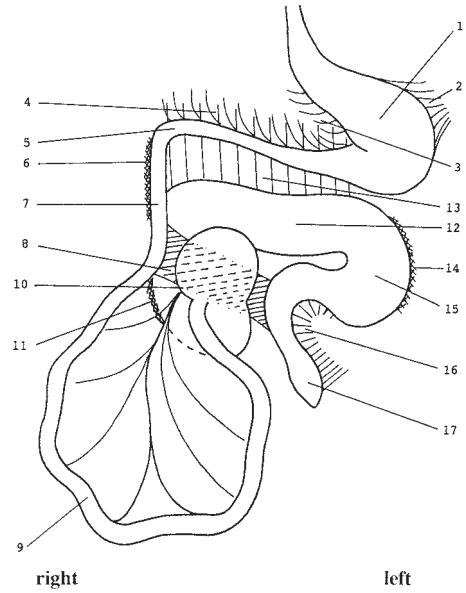

Fig. 3.15 Attachments and mesenteries of the intestinal tract of a Greek tortoise (*Testudo hermanni*): ventral view, schematic.
(1) Stomach
(2) Short ligament fixing the stomach on the left side of the carapace
(3) Hepatogastric ligament (partly fused with the mesocolon of the transverse colon)
(4) Hepatoduodenal ligament (fused with the mesocolon of the transverse colon)
(5) Cranial duodenal flexure
(6) Fusion of the serosa of the descending duodenum with the dorsal peritoneal sheath
(7) Descending duodenum
(8) Plica duodenocolica
(9) Jejunum and ileum
(10) Caecum
(11) Fusion of the serosa of the ascending colon with the dorsal peritoneal sheath (septum horizontale)
(12) Transverse colon
(13) Mesocolon of the transverse colon
(14) Fusion of the serosa of the descending colon with the dorsal peritoneal sheath (septum horizontale)
(15) Descending colon
(16) Mesorectum
(17) Rectum
(Courtesy of Jean Meyer)

Small intestine

The small intestine lies in the caudal coelomic cavity and is not well divided into duodenum, jejunum and ileum (Guard 1980). Beginning at the pylorus, the duodenum runs directly caudal to the liver and is connected to the right liver lobe by the hepatoduodenal ligament. The cranial and caudal pancreoduodenal arteries provide afferent blood supply and venous blood joins the portal vein through the duodenal vein (Ashley 1955).

The descending part of the duodenum is intimately linked to the dorsal pleuroperitoneal membrane which fixes this part of the small intestine in position. The rest of the duodenum, jejunum and ileum are suspended on the *mesenterium proprium*, allowing more or less free movement in the coelomic cavity. The duodenum enters the caecum medially and the junction shows a distinct muscular valve (Guard 1980). The blood supply to the duodenum comes through the cranial mesenteric artery (Ashley 1955; Guibé 1970).

The relief of the mucosal surface is complex at the proximal end of the duodenum, but loses structure further distally (Parsons & Cameron 1977). The mucosa is composed of simple columnar epithelium. Regeneration of the intestinal epithelium takes about eight weeks in *Chrysemys picta* at a temperature of 20–24°C (Wurth & Musacchia 1964).

Large intestine

The large intestine begins at the caecum, which lies in the right caudal quarter of the coelomic cavity. The caecum is not a distinct organ but a widening of the distal colonic wall (Ashley 1955; Guibé 1970). The large intestine can be divided into ascending, transverse and descending colons.

In *Testudo hermanni* the ascending and descending parts are attached with a very short ligament to the dorsal pleuroperitoneal membrane, whereas the transverse part is more loosely connected to the stomach via the *mesogastrium*. This allows the transverse part to move in a dorsoventral direction. Because of this, heavy ingested material (sand, stones etc.) often becomes entrapped in this region as a result of gravity (Meyer 1996).

According to Guibé (1970), the blood supply to the small and large intestines comes through a common mesenteric stem.

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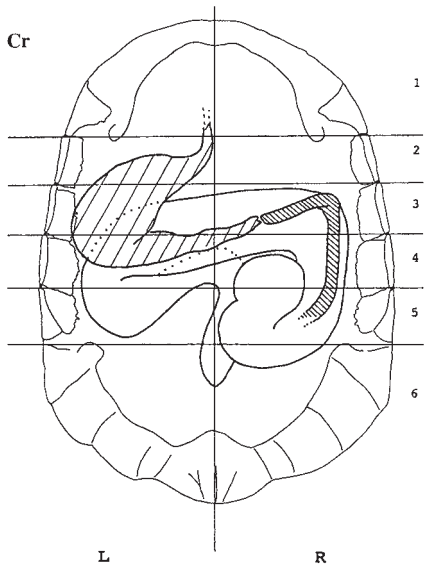

Fig. 3.16 Position of the different parts of the intestinal tract in reference to the bony plates in the female Greek tortoise (*Testudo hermanni*). In the male tortoise the whole digestive tract is placed further caudally due to the lack of the large reproductive tract. (Dorsal view, schematic.)
Coarse hatching = stomach
fine hatching = descending duodenum
outlined = large intestine
Cr = Cranial
(Courtesy of Jean Meyer)

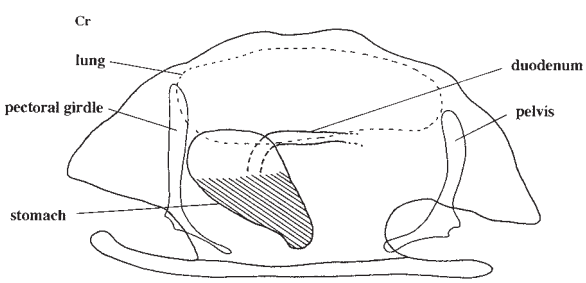

Fig. 3.17 Position of the stomach and descending duodenum in a laterolateral view (schematic).
Cr = Cranial
(Courtesy of Jean Meyer)

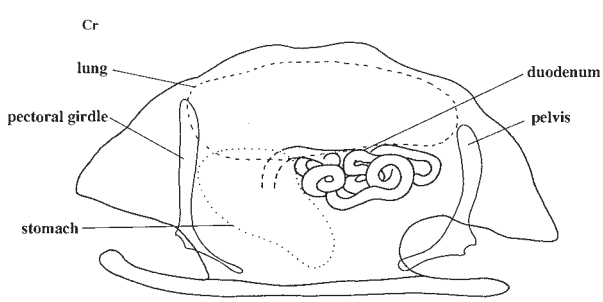

Fig. 3.18 Position of the small intestine in relation to the lung field and the stomach in a laterolateral view (schematic).
Cr = Cranial
(Courtesy of Jean Meyer)

Ashley (1955), however, describes a separate mesocolon. The relief of the mucosa depends widely on the filling of the organ. The mucosal epithelium is similar to that of the duodenum with the exception that it is made up of a larger number of glandular

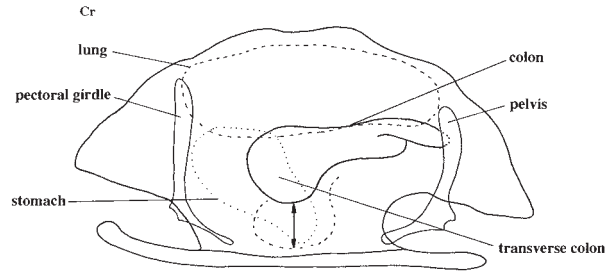

Fig. 3.19 Position of the large intestine in relation to the lung field and the stomach in a laterolateral view (schematic). Note the dorsoventral mobility of the transverse colon (arrows).
Cr = Cranial
(Courtesy of Jean Meyer)

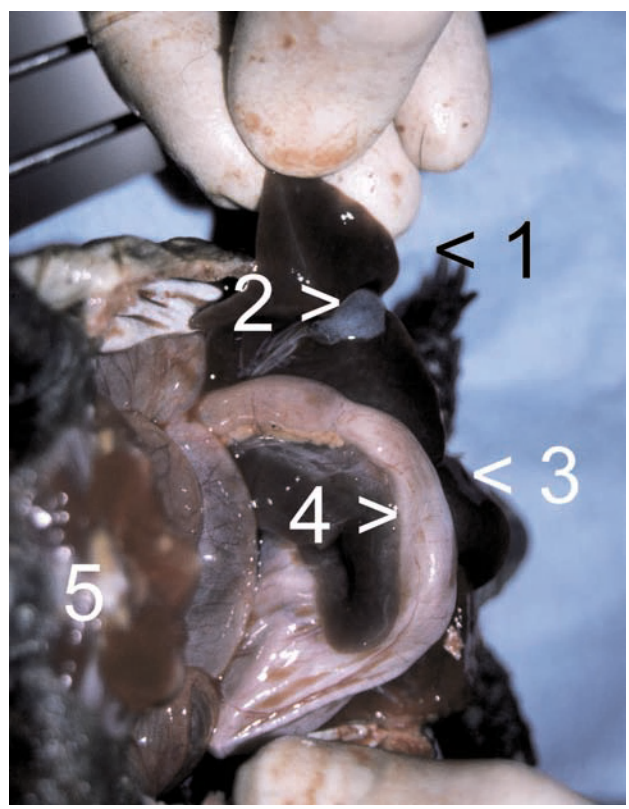

Fig. 3.20 Liver and gall bladder of *Testudo hermanni.*
(1) Right ventral lobe of liver (dorsal view)
(2) Gall bladder seated within right liver lobe
(3) Central lobe of liver
(4) Cranial duodenal flexure attached to the dorsal aspect of the central and right lobe of the liver
(5) Pericardium

cells. The total mucosal surface of the digestive tract of *Testudo horsfieldi* is suggested to be 51000 mm^2 (Skoczylas 1978).

Cloaca

In reptiles, the cloaca (the most distal portion of the conjoined urogenital and digestive tracts) is subdivided into three sections, *coprodeum*, *urodeum* and *proctodeum*. Subdivision is least pronounced in chelonians. The cloaca is described in greater detail later in the section concerning urogenital physiology and anatomy, and representative diagrams are given there.

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Table 3.3 Microscopic comparison of active and hibernating hepatic tissues of *Testudo graeca*.

	Active (June)	Hibernation (February)
Hepatocytes	Hepatocytes are large and relatively similar to each other.	Hepatocytes are smaller than those of non-hibernating animals with greater variations from cell to cell. Degenerating hepatocytes are present. Haemosiderin- and melanin-containing masses are more abundant during hibernation.
	Cytoplasm contains large, well-developed organelles suggestive of high metabolic activity.	Cytoplasm contains fewer, less well-developed organelles. Rough endoplasmic reticulum is reduced and smooth endoplasmic reticulum is abundant.
	Glycogen rosettes and lipid droplets are abundant and located throughout cytoplasm.	No glycogen or lipid droplets present.
Kupffer cells	Only occasional Kupffer cells in sinusoidal walls with cytoplasm containing few organelles and lipid vacuoles.	Kupffer cells more abundant.

The colon ends in the coprodeum. A distinct fold separates the colonic opening from the urodeum, which lies cranially and contains the openings to the ureters, the oviducts or vas deferens and the bladder. The proctodeum receives the outflow of the bladder, urodeum, coprodeum, genital organs and ureters. The proctodeum is the most caudal part and opens to the outside world at the vent.

Liver

Hepatic anatomy

The liver lies centrally within the chelonian coelomic cavity and covers the width of the coelomic cavity behind the heart. It is a large organ, incompletely divided into lobes and with a distinct, small gall bladder at the caudal border on the right side. It has two dominant ventral lobes and the gall bladder lies peripherally in the right of the two. As no diaphragm is present, there are differences in its relationships to the heart, lungs, stomach, intestines and other viscera when compared to higher vertebrates. Normal liver texture and colour are similar to that of other vertebrates.

The microstructure of the liver of *Testudo graeca* is well described by Ferrer *et al.* (1987). Hepatocytes are arranged in tubules and trabeculae as in higher vertebrates, but there is a less lobular arrangement. Frye (1991a) also describes the microscopic architecture of the reptilian liver and points out that a basic lobular arrangement is sometimes present, though less than in higher vertebrates, and that the hepatocellular parenchyma often contains large amounts of randomly-distributed melanin.

Hepatic function

The chelonian liver performs functions similar to those of the liver of higher vertebrates. It is central to lipid, glycogen and protein metabolism. It contains the enzyme pathways responsible for nucleotide/purine degradation to excretion end products such as uric acid, and acts as a major fat body and energy store. As in other higher vertebrates, the chelonian liver is involved in most homeostatic processes. It is likely that the liver houses many synthetic pathways for important physiological activities such as the partial activation of vitamin D (calcitriol).

The functions of the chelonian liver in many species are affected by annual events such as hibernation, when large amounts of fat may be temporarily stored, and reproduction in females, when vitellogenesis and increased protein synthesis prevail. In both situations the normal liver may be large and altered in both colour (pale) and texture (soft). Changes associated with season, reproductive status and the metabolism of hibernation must be differentiated from primary hepatic disease and changes associated with anorexia and other disease conditions.

Ferrer *et al.* (1987) sampled livers of wild tortoises, *Testudo graeca*, that were either hibernating (February) or free ranging (June) and then compared these using both light and electron-microscopy. It is assumed that their findings are representative of normal liver structure in this species (Table 3.3).

Seasonal changes in liver structure and functions are consistent with decreased synthesis, storage and release of liver products during hibernation and a converse increase as the metabolism is raised in the active, non-hibernating state. This implies that tortoises exposed to conditions able to trigger the hibernating state (thought to be temperatures near or below 15°C and poor illumination) are likely to have impaired liver function.

Bile acids

Bile acids produced by chelonians have chemical characteristics not found in any other vertebrates (Skoczylas 1978). These characteristics support a theory of very early separation of turtles and tortoises from the main trunk of reptiles during evolution. This may have significance if one tries to use liver function tests developed for other vertebrates on the basis of their bile acids on chelonian species.

Bile acids help in digestion, in conjunction with the lipases, by forming micellar solutions of triglycerides. These solutions are then subject to absorption. Bile salts facilitate the absorption of fatty acids, monoglycerides, cholesterol, fat-soluble vitamins, calcium phosphate and possibly other different cations (Skoczylas 1978).

Fat bodies

The fat bodies of reptiles are described by Derickson (1976) and Elkan (1980). Fat bodies are more common in species from

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temperate climates than tropical climates (Elkan 1980), and their functions are poorly determined. They are divided by some authors, such as Rollinat (1934), into those serving metabolic needs and those providing structure, with the latter varying little with metabolic needs. They vary in size in relation to starvation (Belkin 1965), reproduction, hibernation and illness (Jackson 1980b). Fat bodies may also be involved in water conservation, especially in species from tropical climates (Elkan 1980). Derickson (1976) explores the seasonal alterations in lipid metabolism of reptiles in depth.

Ferrer *et al.* (1987) demonstrate seasonal changes in liver structure and functions in *Testudo graeca* consistent with decreased synthesis, storage and release of liver products during hibernation and a converse increase, as the metabolism is raised, in the active, non-hibernating state, with significant fat reserves expended during hibernation.

Pancreas

In most chelonian species the pancreas is situated adjacent to the proximal part of the duodenum. According to Ashley (1955), this long, slender, pale gland is connected to the duodenum by a short pancreatic duct. Van der Hage (1985) found a larger number of ducts in many *Testudo* spp. and some *Malaclemys* spp.

The blood supply to the pancreas is guaranteed by branches of the coeliac artery which drain into veins of the hepatic portal system (Ashley 1955). The exocrine and endocrine physiological functions of the chelonian pancreas are similar to that of other vertebrates.

Pancreatic secretions are characterised by two main components:

- digestive enzymes, such as amylase, trypsin, chymotrypsin, carboxypeptidase, elastase, lipase, ribonuclease and chitinase;
- alkaline secretions that neutralise acidic gut contents and form favourable conditions for pancreatic and intestinal enzymes.

Mauremys caspica feeds upon crustaceans and consequently secretes chitinase, while the herbivorous species *Testudo hermanni* lacks this enzyme. The concentrations of proteolytic enzymes in reptiles are similar to the values for other vertebrates. More details about the biochemical characteristics of these enzymes in different chelonian species are available in Skoczylas (1978).

The exocrine pancreas seems to be under the hormonal influence of secretin, as in other vertebrates. The synthesising abilities of the pancreas allow it to adapt the enzymatic composition of pancreatic juice to the diet. The activity of amylase is temperature dependent with higher activity at higher temperatures (Kenyon 1925).

Digestive physiology

Chelonians may be carnivorous, omnivorous or herbivorous. Several commonly-proposed 'herbivorous' species have been observed to be opportunistically omnivorous when fed inappropriate diets in captivity (Frye 1991a). However, this does not mean they will remain in good health if offered a regular omnivorous diet (Bone 1992). Dietary preferences for most chelonians encountered in captivity are described in texts such as Ernst & Barbour (1989) and a guide is given later in the nutrition section of this book.

Table 3.4 Chelonian digestive enzymes.

Organ	Digestive enzyme
Stomach	amylase, pepsin, trypsin, chitinase, chitobiase
Pancreas	amylase, ribonuclease, trypsin, chymotrypsin, carboxypeptidase A, chitinase
Intestine	proteinase, invertase, amylase, maltase, chitobiase, trehalase, isomaltase, sucrase

Digestive enzymes

Various digestive enzymes are secreted by the stomach, pancreas and intestines of both omnivorous and herbivorous chelonians (Dandifrosse 1974) (Table 3.4). These are also described further in a review of the physiology of digestion (Skoczylas 1978).

Importance of temperature and the appropriate temperature range (ATR)

Dawson (1975) and Skoczylas (1978) stress the significance of adequate temperature provision in the process of digestion. Rates of decomposition of ingested elements are determined by temperature. Specifically, the amount and the activity of secreted enzymes (Wright *et al.* 1957; Riddle 1909), and the absorptive processes in the gut mucosa, are a function of temperature, with no digestion taking place below 7°C and extremely slow digestion between 10–15°C (Guard 1980).

Fox (1961) showed that the transport of monosaccharides through the mucosa peaks at 20°C and the process is reduced at high (37°C) or low (2°C) temperatures. Digestion is greatest within the appropriate temperature range (ATR) and is reduced at higher temperatures. At high temperatures gastric HCl production is reduced but pepsinogen secretion remains constant, therefore this enzyme can't work at its optimum pH. Cell membrane permeability of the gut mucosa is altered at different temperatures, changing the absorptive processes of glucose and amino acids as well as substrate affinity to trypsin (Guard 1970). Riddle (1909) showed that digestive rate in chelonians is subject to seasonal fluctuations, being higher in summer than in spring. Gilles-Baillien (1970) found seasonal differences in absorption rates of L-alanine in *Testudo hermanni* with significant decrease in September. During hibernation, high intracellular potassium levels seemed to limit L-alanine absorption (Gilles-Baillien & Schoffeniels 1961).

It is suggested that thermal inertia associated with size is beneficial to herbivorous reptiles and helps maintain effective digestive function. It goes some way to explaining the size of giant terrestrial species such as the Galapagos tortoise (*Chelonoidis nigra*) and aquatic species such as the leatherback turtle (*Dermochelys coriacea*). Generally the absorptive capacity for lipids, sugars, amino acids and ions per mucosal surface unit is smaller for reptiles than for mammals, but comparable in mechanism (Guard 1970).

When temperatures are below a critical level, putrefaction of ingesta dominates the process of digestion. It is extremely important in hibernating species and in species being kept at latitudes where supplementary heat is required to compensate for temperatures below those to which a species is evolutionarily accustomed

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(Skoczylas 1978). The absorption of toxic putrefaction products, as a result of inadequate temperatures for digestion, may account for some hibernation mortality and for syndromes such as peri-hibernation hind limb paresis common in United Kingdom captive Mediterranean tortoises (*Testudo* spp.). Food-derived toxins may affect the normal functioning of nervous and other tissues, although work to investigate such events does not appear to have been undertaken. This author (SM) advises that chelonians should be maintained within their ATR wherever possible, unless preparations are being made for hibernation. In this situation appropriate dietary control and pre-hibernation temperature management are essential. This is described elsewhere in this book.

Gut motility and ingesta passage time

Skoczylas (1978) suggests that gut passage time is a reflection of gastric motility, which in turn is a reflection of both the health and the ambient temperature of a reptile. Peristalsis facilitates the transport of ingesta through the digestive tract, mixes gut contents with digestive enzymes, breaks up food particles, transports nutrients to the absorptive mucosa and allows elimination of indigestible material and metabolites by defecation. In poikilothermic animals this process is largely dependent upon ambient temperature. Fox & Musacchia (1959) showed that *Chrysemys picta* kept at 5°C did not show any gastric emptying for four days, whereas at room temperature the stomach only contained small amounts of the meal after 24 hours.

Patterson (1933) and Hukuhara *et al.* (1975) found that chelonian gut contractions are not continuous, but occur in series. In *Chelydra serpentina* such series last 5.5–6 hours and are separated by several hours of rest. This is important when considering radiographic contrast studies. Gastric contractions start at the cardia, run down the gastric wall in intervals of 21–31 seconds and then end at the pylorus. Vagal impulses control these contractions. Gastric peristalsis is inhibited by parasympatholytic drugs. This process is also temperature dependent, as intestinal muscle contractions appear to respond poorly to vagal nerve stimulation at temperatures below 10°C (Wright *et al.* 1957).

Contractions of the small intestine occur at intervals of approximately 45 seconds (Skoczylas 1978). The large intestine shows two different kinds of peristalsis. The first type begins at the caecum at low speed (0.15–0.5 mm/sec) and ends at the coprodeum. This is generally followed by defecation. The second type is an antiperistalsis which starts at the coprodeum at intervals of 18–25 seconds and propagates cranially for 2–3 cm. Antiperistalsis allows urine to be shifted into the caudal parts of the colon where water and ions can be reabsorbed (Guard 1980).

Gut passage time is inversely proportional to particle size (in the range 2–10 mm). Passage time appears shortest in omnivorous species (*Trachemys* spp.) and longest in herbivorous species, with carnivorous species in between. Radiographic and sectional studies have showed that in omnivorous and carnivorous species the stomach is the longest hold-up, whereas in herbivorous species this appears to be the caecum and proximal colon (Guard 1980):

- gut passage time of the Galapagos tortoise *Chelonoidis nigra* is given as 7–20 days by Rick & Bowman (1961) (temperature not given);
- Lawrence & Jackson (1982) suggest that in Mediterranean terrestrial chelonians (*Testudo* spp.) gut passage time relates to fibre content and moisture as well as temperature. A succulent diet had a passage time of 3–8 days (mean 6.5) and a coarse diet 16–28 days (mean 23) (maintenance at 28°C). These figures are similar to others quoted for *Chelonoidis nigra*;
- an anecdotal report suggests that diarrhoea results in a decreased gut passage time of 3–5 days (Lawrence & Jackson 1982);
- gut passage time should be considered when oral dosing tortoises and assessing the movements of intestinal foreign bodies. Lawton (1997) advises serial radiography and knowledge of gut passage time when monitoring the passage of gastroliths;
- Meyer (1998) showed the influence of temperature on passage time by using Gastrografin® as a contrast agent in *Testudo hermanni*. The mean total transit times were 2.6 hours (range 1.5–4 hours) at 30.6°C, 6.6 hours (range 3–8 hours) at 21.5°C and 17.3 hours (range 8–24 hours) at 15.2°C. The absence of gastric contractions for 24 hours in one tortoise showed that gut contractions could be interspersed by periods of rest as described earlier.

Herbivorous tortoises utilise hindgut fermentation, relying on symbiotic microbial digestion of plant material. In some terrestrial species, colonic *Oxyurid* spp. (pinworms) may also assist. Such species have significantly longer gastrointestinal transit times than carnivorous species. The ratio of alimentary tract length to whole body length is greater in herbivorous reptile species than omnivorous species. The large intestines of herbivorous species tend to have a greater volume than those of omnivorous species (Skoczylas 1978).

Ingestion of non-food material

Ingestion of soil, sand, stones or bone is seen frequently in captive tortoises and has also been documented in free-ranging chelonians. Although the reason for this behaviour is unknown, it may serve some mechanical digestive function (Skoczylas 1978). In some situations lithophagy appears to be behaviourally and possibly physiologically driven. Esque & Peters (1994) suggest that such behaviour may assist maintenance of gut pH, detoxify plant toxins, control intestinal parasites, or maintain correct beak shape. Vitellogenesis may be associated with ingestion of white material such as bone, small white stones and broken china, and this appears to coincide with increased metabolic demands for calcium.

Normal chelonian gut flora

Few studies have been conducted to investigate the normal gut flora of chelonians. Most of what is known about the microflora and protozoans of the reptilian alimentary tract is described by Skoczylas (1978). Information on commensal, symbiotic and pathogenic micro-organisms can also be found in Barnard (1986).

An investigation into the bacterial faecal flora of clinically healthy Mediterranean tortoises (*Testudo* spp.) was carried out by Sunderland & Veal (2000). They isolated *Aerococcus* spp., *Enterococcus* spp., *Micrococcus* spp., *Rhodococcus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Acinetobacter* spp., *Actinobacillus* spp., *Citrobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Escherichia* spp., *Klebsiella* spp., *Morganella* spp., *Pasteurella* spp., *Proteus* spp., *Pseudomonas*

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spp., *Salmonella* spp., *Serratia* spp., *Shigella* spp., *Yersinia* spp. and further unidentified species from the faeces of normal United Kingdom captive chelonians. An earlier study by Smith (1965) tried to isolate bacteria from different parts of chelonian intestines, but his methods were more appropriate to the investigation of organisms present in endotherms and it has been suggested that only 1%–10% of bacterial isolates present may actually have been noted Donaldson (1968). *Bacteroides* spp. were identified by Smith (1965) in the rectal contents of a Mediterranean *Testudo* indicating the ability of the intestinal bacterial microflora to act symbiotically in the degradation of ingesta in herbivorous species (Skoczylas 1978). Enzymes released by symbiotic intestinal bacteria and yeasts are assumed to be significant in the decomposition of items of plant origin such as cellulose.

Cooper (1981) describes *E. coli*, *Proteus* spp., *Aeromonas* spp. and *Pseudomonas* spp. as common isolates from cloacal swabs and gut contents of normal chelonians. Cooper also suggests that normal gut flora of reptiles may be altered by changes in ambient temperature. MacDonald (1998) investigated the oral flora of healthy and diseased United Kingdom captive terrestrial chelonians and concluded that, where chelonians are maintained in groups, oral flora is often similar. This would suggest that oro-oral contamination and oro-faecal contamination are potentially commonplace and that bacteria and diseases may be transmitted in these ways.

Coccidia, amoebae, *Cryptosporidium* spp., flagellates such as *Trichomonas* spp., ciliates such as *Balantidium* spp., ascarids, *Proatractis* spp., oxyurids (pinworms), trematodes (flukes) and yeasts have all been identified in faeces from diseased chelonians. It is not always easy, however, to categorise such organisms as either normal or pathogenic. In many situations it must be argued that moderate numbers of many of these organisms are beneficial to the host and break down gut contents. In the wild, gut burdens of such organisms are generally mild and cause no serious trouble. However, the conditions of captivity encourage self-reinfection, burdens rise and pathological changes may occur. The clinical pathology, therapeutics and problem-solving sections later in this book give advice regarding how burdens of these organisms are appropriately identified and managed in captivity.

Organisms associated with disease in man, such as *Yersinia enterocolitica*, *Candida albicans*, *Salmonella* spp., *Vibrio* spp. and *Cryptosporidium* spp. have all been reported as existing in the digestive tract of clinically normal chelonians. Of the twenty-two genera identified by Sunderland & Veal (2000) in faeces from normal captive chelonians in the UK, many bacterial isolates are potential zoonoses, including *Citrobacter, Klebsiella, Morganella, Serratia, Salmonella, Staphylococcus, Streptococcus* and *Yersinia*. This highlights the necessity for hygienic conditions when dealing with tortoises, and underlines the responsibility of the veterinarian to inform owners of the risks involved in handling them.

In addition to potential pathogens, one would always expect a wide variety of bacteria to inhabit the intestines of any healthy chelonian. Further details of these organisms can be found in the appendices in table form. Where only a narrow range of bacteria are noted upon faecal microscopy and culture, it is possible that a variety of influences have altered intestinal flora, and this may in turn affect the health of the chelonian, as described later.

URINARY SYSTEM

Urinary anatomy

Chelonians have two kidneys. These are located in the caudal retrocoelomic cavity and they are often in close association with the carapace, just cranial to the pelvic girdle. The caudal lung fields and carapace are dorsal to the kidneys (Figs 3.21–3.22). The reptilian kidney has an advanced metanephric structure typical of

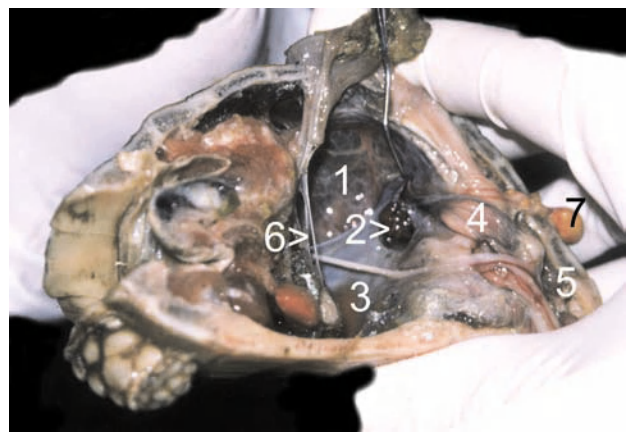

Fig. 3.21 Further dissection revealing the coelomic cavity and kidney. A small hole has been incised in the caudal coelomic cavity to reveal the right kidney (2).
(1) Lung revealed by removal of a portion of the horizontal membrane/coelomic membrane
(2) Right kidney revealed in its retrocoelomic location through a small incision in the coelomic membrane
(3) Intact coelomic membrane
(4) Cloaca displaced caudally
(5) Cloaca-associated viscera (bladder, oviduct and large intestine) displaced caudally
(6) Mesentery suspending small intestine reflected caudally outside the coelomic cavity

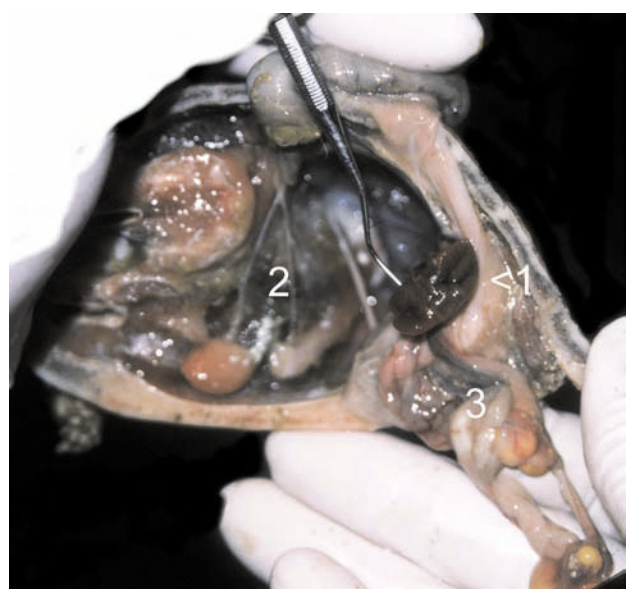

Fig. 3.22 Further dissection revealing the right kidney (arrowed 1).
(1) The kidney is exteriorised in its entirety
(2) Mesentery suspending small intestine
(3) Cloaca and associated viscera (bladder, oviduct and large intestine) displaced caudally

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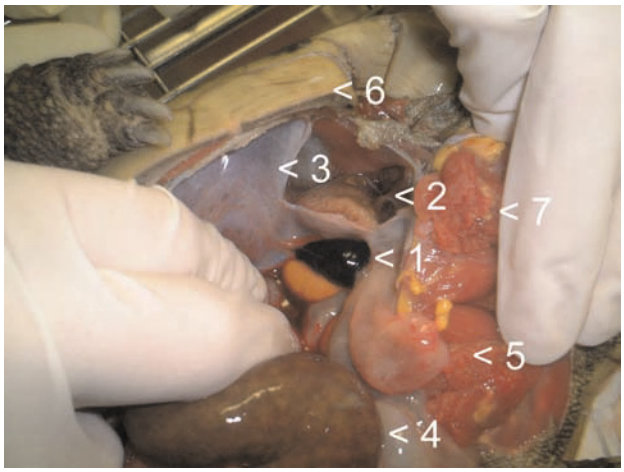

Fig. 3.23 Urogenital anatomy of male *Testudo hermanni* demonstrating extensive renal gout deposition.
(1) Testicle (pale) attached to epididymis (darker colour)
(2) Kidney (abnormal in shape and colour) lying retrocoelomically, viewed through incised window of coelomic membrane
(3) Coelomic membrane (incised)
(4) Liver lobe reflected cranially
(5) Remnants of urodeum with bladder and cloacal structures removed
(6) Bridge
(7) Pelvic musculature (trimmed)

higher vertebrates, but lacks a loop of Henlé and a renal pelvis. The primary unit is the nephron, and this consists of a glomerulus, a short, slender neck, a thicker and longer proximal tubule, a short, thin, intermediate segment and a distal tubule (Dantzler 1976). Solomon (1985) describes the morphology of the kidneys of marine turtles. A renal portal system exists in all chelonians and the significance of this is described earlier.

In the male terrestrial chelonian, the kidneys are closely associated with the paired craniomedial male gonads (testes), which are easily mistaken for kidneys at post mortem or exploratory coeliotomy (Mader 1997) (Figs 3.23–3.24). In female tortoises, the kidneys are positioned behind the coelomic membrane and in front of the pelvic girdle, with oviducts and ovaries further forward within the body of the coelomic cavity (Figs 3.25–3.26).

Bilateral ureters enter the urodeum of the cloaca dorsally, at approximately 10 and 2 o'clock positions, when viewed in transverse cross section. The structure and function of the cloaca are also described elsewhere in this book. The urodeum allows urine either to be passed caudally into the proctodeum to be mixed with faeces there, into the coprodeum and colon through antiperistalsis or cranially into the bladder. In some semi-aquatic chelonians two small accessory bladders may also be present, attached to the urodeum. A short urethra enters the bladder through the mid-ventral floor of the urodeum (Mader 1997).

The kidneys lie beneath the carapace at the caudal border of the lungs. They produce urine that is hypotonic or isotonic to blood and actively excrete uric acid. The ureters empty into the urodeum whence urine may be diverted into the capacious, thin-walled bladder (at times when water needs to be resorbed) (Figs 3.27–3.29) or shunted directly into the proctodeum and then voided. The bladder wall is lined with ciliated cells and secretes mucus, which facilitates the handling of urate crystals. The coprodeum receives faecal material from the colon and also empties into the common proctodeum.

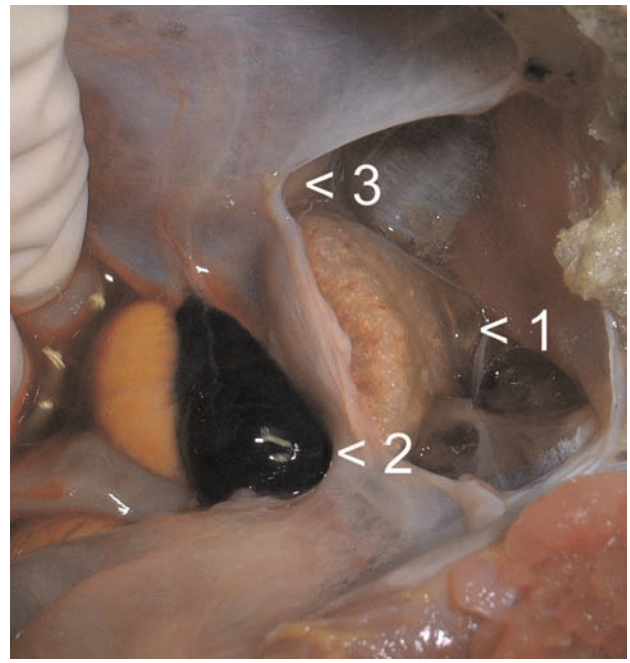

Fig. 3.24 Close up of urogenital anatomy of male *Testudo hermanni* demonstrating extensive renal gout deposition (Fig. 3.23).
(1) Retrocoelomic kidney. This kidney is extensively affected by gouty deposition and is abnormally pale, granular and significantly enlarged
(2) Testicle (pale) and epididymis (darker) within the coelomic cavity
(3) Incised coelomic membrane showing the division between the kidney and testicles and their coelomic membrane relationship

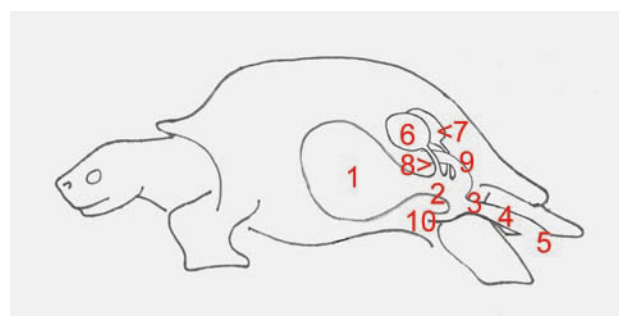

Fig. 3.25 Schematic cross sectional diagram of urogenital anatomy of a mature *Testudo*. Representations of major urogenital structures are shown.
(1) Bladder
(2) Urodeum
(3) Coprodeum
(4) Proctodeum
(5) Vent
(6) Left kidney (NB retrocoelomic)
(7) Right kidney (NB retrocoelomic)
(8) Left ureter
(9) Rectum (NB coelomic)
(10) Reproductive organ outlet (i.e. oviduct or vas deferens)

Urinary physiology

Renal physiology varies from species to species depending upon environmental demands. An understanding of these adaptations is fundamental to chelonian medicine. Terrestrial chelonians from arid environments tend to be *uricotelic* (excreting nitrogenous waste mainly as uric acid and urates) or *ureo-uricotelic*

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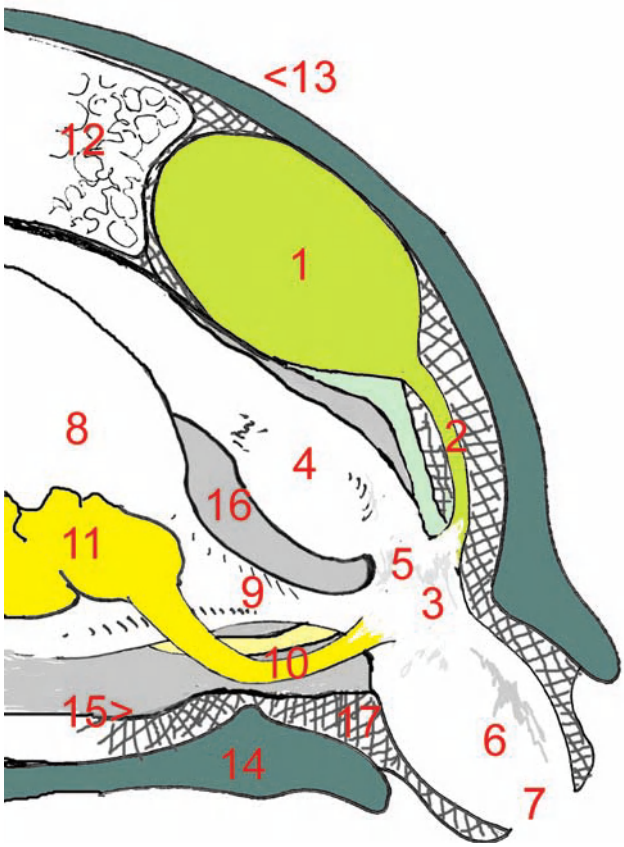

Fig. 3.26 Schematic cross sectional diagram of urogenital anatomy of a mature *Testudo*. Representations of major urogenital structures are given.
(1) Right kidney
(2) Right ureter (the left is represented with dotted lines)
(3) Urodeum
(4) Large intestine
(5) Coprodeum
(6) Proctodeum
(7) Vent
(8) Bladder
(9) Neck of bladder entering urodeum
(10) Paired ducts of genital organs
(11) Genital organ (oviduct/testes)
(12) Lung tissue
(13) Carapace
(14) Plastron
(15) Coelomic membrane extending into the septum horizontale
(16) Shaded area representing the coelomic cavity
(17) Hatch shading representing muscles and fascia

(excreting a combination of uric acid and urea), whereas semi-aquatic species are likely to be *amino-ureotelic* (excreting a combination of ammonia and urea).

The functions performed by reptilian kidneys include osmoregulation, fluid balance regulation, excretion of metabolic waste products and the production of hormones and vitamin D metabolites (Moyle 1946; Dantzler & Schmidt-Nielson 1966; Dantzler 1976; Minnich 1982; Mader 1997). In the literature, production of erythropoietin and activation of Vitamin D precursors by the kidneys are not specifically described, but may be similar to higher vertebrates (SM: personal assumption).

In some species of chelonians, especially terrestrial tortoises, both the kidneys and the bladder appear to be involved in electrolyte excretion and fluid balance (Jorgensen 1998) (Fig. 3.25). In the Californian desert tortoise *Gopherus agassizii* ureteral urine

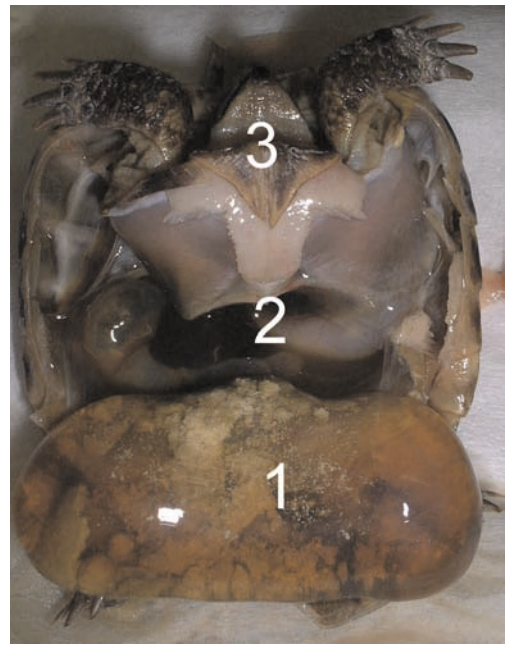

Fig. 3.27 Juvenile *Geochelone sulcata* showing potential size of bladder.
(1) Bladder (distended and reflected caudally)
(2) Coelomic cavity occupied by bladder prior to its exteriorisation
(3) Head and cranial tortoise for reference

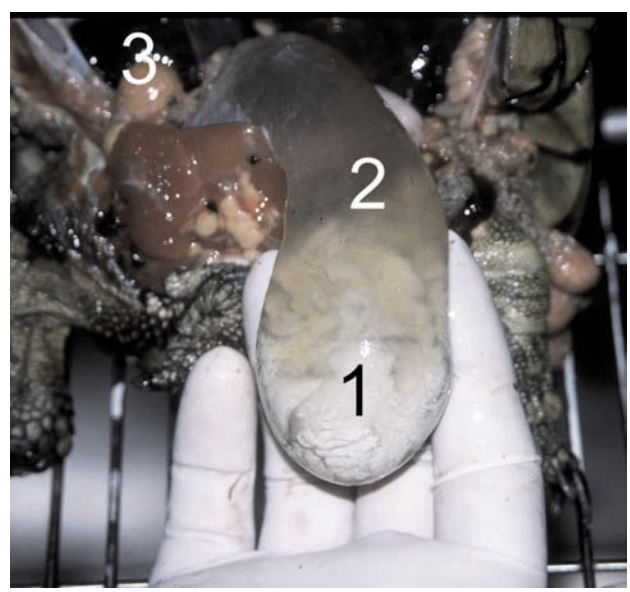

Fig. 3.28 Post mortem display of left bladder lobe of a mature female *Testudo hermanni*.
(1) Distended exteriorised bladder lobe containing white potassium urate deposits
(2) Soluble portion of urine within distended bladder lobe. Vessels of the bladder are not apparent because the picture is post mortem
(3) Coelomic cavity

entering the bladder undergoes an equilibration process with other body fluids (Dantzler & Schmidt Nielson 1966). In marine chelonians the salt gland and the urinary tract appear to co-operate in magnesium, sodium and potassium excretion and regulation of fluid balance (Schmidt Nielson *et al.* 1963; Lutz 1996).

According to authors such as Moyle (1946), Dantzler (1976) and Minnich (1982), reptiles are unable to concentrate urine to

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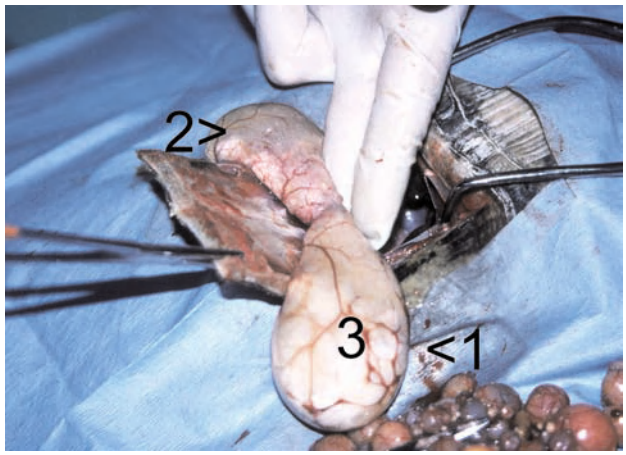

Fig. 3.29 Ante mortem display of the bladder of a mature *Testudo hermanni* during a coeliotomy achieved through a plastron osteotomy.
(1) Left lobe of bladder containing soluble urine portion and insoluble urate precipitate
(2) Right lobe of bladder
(3) The vessels of the bladder are large and extensive (compare this to the bladder in Fig. 3.32)

Table 3.5 The distribution of nitrogenous products excreted by chelonians in different environments (derived from Moyle 1946 and Dantzler & Schmidt-Nielson 1966).

Habitat	Per cent of total urinary nitrogen as		
Species	Ammonia	Urea	Uric Acid
Aquatic	20–25	20–25	5
Semi-aquatic	6–15	40–60	5
Trachemys scripta	4–44	45–95	1–24
Terrestrial			
Kinixys erosa	6.1	61	4.2
Testudo graeca	4.1	22.3	51.9
***Hydrophilus* sp**	6	30	7
***Xerophilus* sp**	5	10–20	50–60
Gopherus agassizii	3–8	15–50	20–50

an osmolarity greater than that of plasma. In *Gopherus agassizii*, Dantzler & Schmidt-Nielson (1966) demonstrated that urine in the renal tubules and ureters is always hypo-osmotic to the blood. Such urine becomes iso-osmotic through equilibration in the bladder. The bladder was therefore suggested to have a fluid and electrolyte regulating function. Hypo-osmotic tubular and ureteral urine reduces the risk of urate precipitation within renal tubules during periods of dehydration in uricotelic chelonians (Minnich 1982).

In chelonians, urinary nitrogen is excreted as a balance of ammonia, urea, uric acid, amino acids, allantoin, guanine, xanthine and creatine (Moyle 1946; Khalil & Haggag 1955; Dantzler & Schmidt-Nielson 1966; Dantzler 1976) (Table 3.5).

Chelonian excretion patterns

There are four chelonian excretion patterns:

- Uricotelism, in which the major urinary excretion products are uric acid and urates;
- Ureotelism, in which the major urinary excretion product is urea;
- Amino-ureotelism, in which the excretion products are a combination of ammonia and urea;
- Ureo-uricotelism, in which the products of excretion are uric acid and urea combined.

Excretion patterns strongly reflect the environment from which an animal comes. Where water must be conserved, uricotelism is more energy efficient than ureotelism. With uricotelism, urates precipitate out of solution under favourable conditions in the bladder and do not need to be actively concentrated in solution. In terrestrial mammals from arid environments, energy is expended in maintaining the renal medullary sodium concentration, which is responsible for resorption of water. Such energy expenditure would represent a considerable demand for a poikilothermic reptile. Ureotelism is practical for chelonians only where water is cheap, and uricotelism is a logical evolutionary response to living in an arid environment where both energy and water are at a premium.

Chelonians from environments where water is plentiful generally excrete combinations of ammonia and urea. Semi-aquatic turtles, such as *Trachemys scripta*, are predominantly amino-ureotelic. Urea is highly water soluble and readily crosses biological membranes. It is difficult for chelonians to concentrate and must therefore be voided with significant amounts of water.

Chelonians from environments where water is relatively scarce, or where hibernation may occur for several weeks without fluid replenishment, tend to excrete uric acid in large proportions. Terrestrial chelonians, such as *Gopherus agassizii*, are predominantly uricotelic or ureo-uricotelic. Compared with urea, uric acid is poorly soluble and can be excreted with minimal associated water. The role of the bladder in the precipitation of urates and re-absorption of water is described later. Khalil and Haggag (1955) found *Testudo kleinmanni* and *Geochelone sulcata* excreted less urea, and more uric acid, when dehydrated. Dantzler (1976) suggested that their results should be interpreted as showing bladder as well as renal influences because they assessed bladder urine.

The urine of marine turtles has varied dramatically between studies. Bjorndal (1979) found little ammonia and significant urea in urine from *Chelonia mydas*, yet Prange & Greenwald (1980) found the opposite. Khalil (1947) found urea in only one out of four turtles he examined. It would appear that excretion products are affected by changes in hydration (Lutz 1996) and are not constant for a given species.

The role of the bladder and lower digestive tract in electrolyte and fluid balance

The bladder appears to play a significant homeostatic role in both terrestrial uricotelic species of chelonians and amino-ureotelic aquatic species (Jorgensen 1998) (Fig. 3.30).

The bladder of the desert tortoise *Gopherus agassizii* is highly permeable to water, urea, ammonia and small ions, but not urates. In this species, Dantzler & Schmidt-Nielson (1966) suggest that if all ureteral urine entered the bladder, then the kidneys would play no role in controlling urine composition with regard to electrolytes and water. They demonstrated the chelonian bladder to have a role in electrolyte excretion, urate precipitation and water

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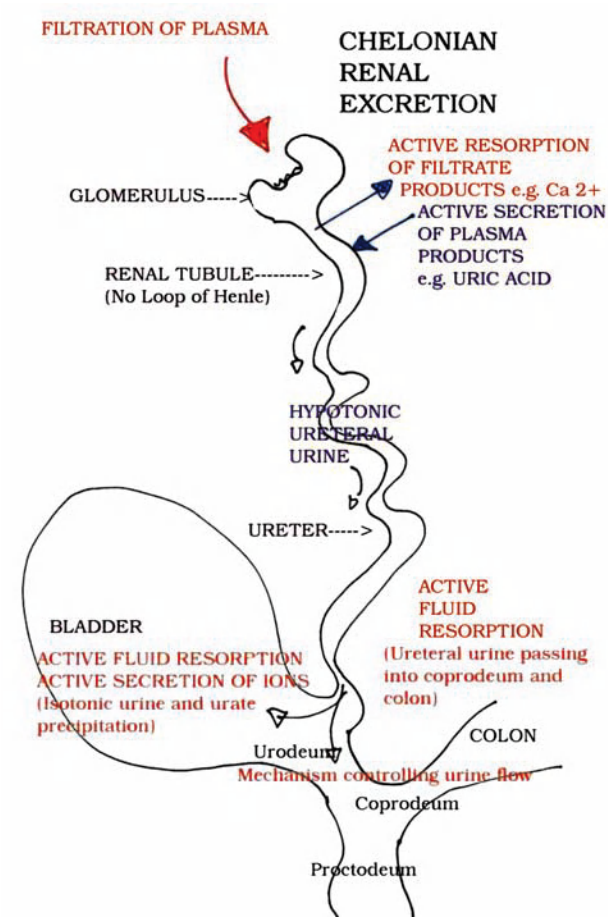

Fig. 3.30 Schematic representation of processes involved in renal excretion of urate and lower urinary tract recovery of water.
This diagram applies to uricotelic terrestrial species.

absorption. This has implications for the use of urine specific gravity or clearance calculations as a measure of renal function, unless samples of ureteral (and not bladder) fluid are used.

In chelonians the potential exists for ureteral urine to be channeled in several different ways depending upon hydration, fluid intake and other factors relating to blood biochemistry. We do not yet know how much ureteral urine entering the urodeum may bypass the bladder, or when and how active ion transport occurs in the lower urinary tract:

- Some ureteral urine entering the urodeum is directed into the coprodeum/proctodeum, away from the bladder. Such urine would be excreted once mixed with the faeces. To an observer this would be recorded as faeces and not urine. Some resorption of fluid may occur in the large intestine/proctodeum;
- Some ureteral urine entering the urodeum is directed into the bladder. Here it equilibrates with plasma with respect to osmolarity and electrolyte composition. Uric acid is precipitated out within the bladder (mainly as potassium urate) and these urates are voided at urination;
- Some ureteral urine entering the urodeum may be excreted directly without alterations to composition.

This results in an excretion mechanism that is able to respond to changing environmental demands.

Dantzler & Schmidt-Nielson (1966) found that the amino-ureotelic semi-aquatic chelonian *Trachemys scripta* did not appear to equilibrate bladder fluid, which remained hypo-osmotic to blood. The uricotelic terrestrial *Gopherus agassizii* converted hypo-osmotic ureteral urine to iso-osmotic bladder fluid. In freshwater turtles, active transport of sodium and chloride ions occurs across the bladder wall in tandem with secretion of hydrogen ions and resorption of bicarbonate ions (Dantzler & Holmes 1974; Stetson 1989).

Bladder equilibration is avoided if urine entering the urodeum bypasses the bladder and is excreted immediately. Storage within the proctodeum, and reflux into the large intestine, may enable equilibration through water resorption (Bentley 1962). This also suggests that fluid losses will increase during periods of diarrhoea, over and above what might normally be expected.

Dantzler & Schmidt-Nielson (1966) suggested that a well-developed sphincter at the neck of the bladder, especially in uricotelic species, might allow ureteral urine to bypass the bladder. It is not known how the flow of urodeal urine into either the bladder or the proctodeum is controlled.

Uricotelic terrestrial tortoises like *Gopherus agassizii* from arid desert environments may use their bladders mainly for water conservation, urate precipitation and electrolyte excretion, as opposed to storing urine for excretion. Dantzler (1976) suggests precipitation and excretion of uric acid and urates in the bladder and cloaca permits the excretion of inorganic ions (like potassium) by the urinary route and prevents unnecessary loss of water.

The bladder of aquatic species, such as *Chelonia mydas*, is presumed to have an osmotic and fluid balancing role. Stetson (1989) demonstrated the regulation of ion transport by dynamic changes in plasma membrane area of turtle bladder by H^+ and HCO_3^- secretion pumps, Na^+ and Cl^- absorption. Stetson suggested that all these mechanisms were independent of each other in operation, and had further influences on other ion levels within the body such as intracellular Ca^{2+}. Prange & Greenwald (1980) demonstrated a urine/plasma concentration ratio greater than one in dehydrated marine turtles and this implied that, in co-operation with the salt gland, urine more concentrated than plasma was produced with involvement of active bladder secretion of salts

Excretion of magnesium, sodium and potassium from salt water and food appears to be through an ocular salt gland (Schmidt-Nielson & Fänge 1958; Holmes & McBean 1964; Schmidt-Nielson *et al.* 1963). Schmidt-Nielson *et al.* (1963) propose that the salt gland and the lower urinary tract act together in marine reptiles to conserve physiologically useful desalinated fluid. In this situation the bladder may help with conservation of useful water. In marine species the bladder may also be used to help with balance and buoyancy and it is also feasible that urine retention may reduce predation from aquatic predators with a well-developed sense of smell.

During states of normal hydration it appears that tortoise urination strongly coincides with drinking and bathing. It is hypothesised that this is an evolutionary mechanism developed to ensure conservation of water in arid environments. Minnich (1982) describes the bladder of *Gopherus agassizii* as a place of water storage and potassium excretion as precipitated urates, with hypo-osmotic ureteral urine becoming iso-osmotic to plasma in the bladder.

Minnich quotes Charles Darwin's observations with regard to *Geochelone nigra*: '*. . . For some time after a visit to the springs, their*

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urinary bladders are distended with fluid, which is said gradually to decrease in volume and become less pure' Darwin (1839).

REPRODUCTIVE SYSTEM

All Chelonians are oviparous. Although many lay soft flexible eggs, most lay eggs with hard, calcified shells that are less malleable than those of other reptile species. Sexual maturity in the wild is generally reached at about 15 years of age in both sexes, but this is greatly influenced by rate of growth and size. Female chelonians typically attain maturity later than males. Some captive-bred tortoises become sexually active very early. Leopard tortoise (*Geochelone pardalis*) males have been known to mate successfully at as young as four years of age (Highfield 1996). Accelerated growth and early maturation like this are not necessarily desirable.

Reproductive anatomy

The male chelonian has a single penis, which is not involved in urination. This protrudes from the floor of the cloaca. Paired testes lie within the coelomic cavity, lying directly cranioventral to the retrocoelomic kidneys (Fig. 3.5). The testes fluctuate in size seasonally.

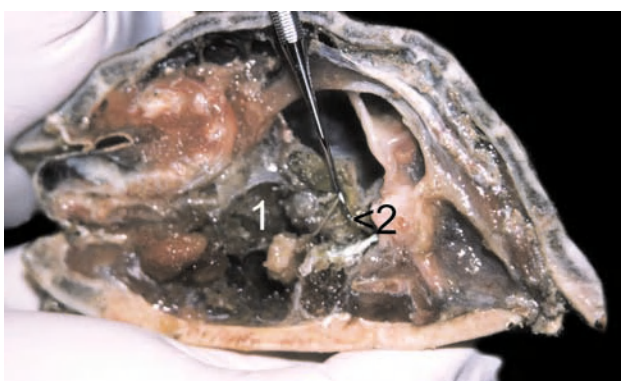

Fig. 3.31 Initial examination of the reproductive tract. The right ovary has been arrowed and remains in its initial location following section of the cadaver. The bladder is collapsed allowing visualisation of other viscera, which would more usually be obscured by it.

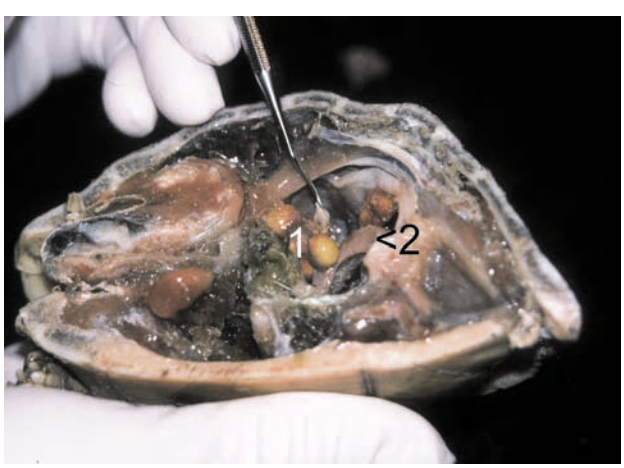

Fig. 3.32 The reproductive tract. The right ovary (1) is displayed and the associated oviduct (2) is revealed as the ovary is gently drawn out.

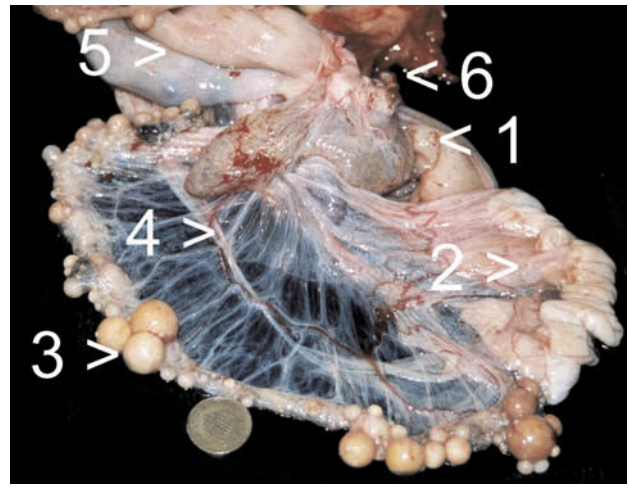

Fig. 3.33 Reproductive anatomy (*Geochelone pardalis*): urogenital tract removed from the animal and displayed.
(1) Urodeum
(2) Oviduct
(3) Active ovary showing follicular development
(4) Vessels of mesovarium
(5) Rectum, coprodeum and proctodeum
(6) The bladder is collapsed and overlying the base of the oviduct and urodeum

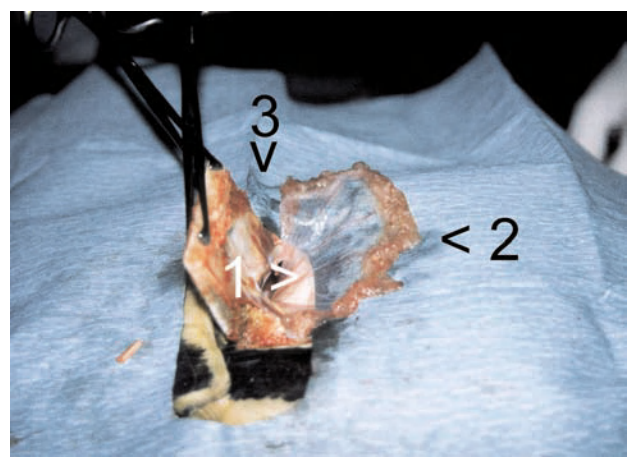

Fig. 3.34 Reproductive anatomy (*Testudo hermanni*): an inactive ovary is displayed intra-operatively through a plastron osteotomy site.
(1) Mesovarium
(2) Inactive ovary with no obvious follicular activity
(3) The osteotomy flap is reflected on its caudal hinge and the animal is in dorsal recumbency.

In the female, the two ovaries are suspended from the dorsal coelomic membrane (Figs 3.31–3.34). At ovulation, ova are released into the long paired oviducts where albumen, membranes and the shell are added. The oviducts enter the urodeum of the cloaca.

Identifying gender

Most chelonians are sexually dimorphic, though external differences are not obvious in juveniles and become more apparent in many species at puberty. It is wise to avoid using external characteristics to determine sex in chelonians less than five years old. In some species it may take as long as ten years before sex

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is apparent. Male divergence away from an essentially female juvenile shape is unlikely to occur before the transition from hatchling/juvenile into adolescent has occurred. Intersexuality has been observed in both wild and captive specimens. If two specimens of the same age and same species are available, we would suggest that they be compared.

There are a number of ways of identifying gender in chelonians (and see Table 1.2):

Cloacal organ

Male chelonians tend to have a large cloacal penis, which may become erect in response to stressful handling. Frye (1991a) describes this as a defence response and says it may be accompanied by urination. He describes the male penis as spade shaped, often heavily pigmented and with a median groove or raphe along which to direct semen during copulation. In *Testudo* spp., the penis may be three or four inches long when erect.

The mere presence of a cloacal organ is not necessarily indicative of tortoise gender. Moderate penis-like protrusions (*clitoral hyperplasia*) are often observed in female tortoises, notably those treated with oxytocin during dystocia and debilitated, hypocalcaemic or oedematous female specimens.

Tail features

Mature males of most species have longer, broader tails than females. Occasionally they are more 'pointy'. The distance from the caudal edge of the plastron to the cloacal opening is generally shorter in females than in males.

Plastron shape

The male plastron is usually curved or indented. Presumably this is an adaptation to assist in mounting and mating. Females may show a moderate *kinetic plastral hinge*, where the transverse sutures between the scutes of the caudal plastron are flexible, producing mobility of the caudal plastron. Frye (1991a) suggests that this is an adaptation to facilitate oviposition. In marine turtles such as the Kemp's Ridley *Lepidochelys kempii*, the male appears to have a softened central plastron area. This is presumed by Owens (1996) to make him receptive to the dorsal carapacial ridge of the female during mating.

Carapace size and shape

Carapace shape may be suggestive of gender. Various adaptations appear to accommodate the development of uterus, follicles and eggs within female tortoises. Adult males are often smaller than females. In some species, such as *Testudo hermanni*, females grow to sizes that are rarely achieved by males. In many species, such as *Geochelone carbonaria*, males are thinner than their typically broader females. Again this is presumably to increase egg-carrying potential. Owens (1996) records the dorsal carapace of mated female sea turtles as potentially scarred as a result of the action of the male flipper claw during previous mating.

Species-specific secondary sex characteristics

Secondary sex characteristics may be species specific:

- In some *Terrapene* spp. the eye colour is bright red in mature males and a yellow brown in mature females;
- Mature males of some semi-aquatic species such as *Trachemys scripta* show greatly elongated claws on their forelimbs;
- Growth of the curved front claws of marine turtles, such as the Kemp's Ridley (*Lepidochelys kempii*), used to grip females when mating, also appears to be under the control of testosterone and are curved and elongated in male turtles (Owens 1996);
- Head coloration and markings may differ between male and female chelonians. In some semi-aquatic species head coloration and markings may differ during the breeding season. In species, such as the elongated tortoise (*Indotestudo elongata*), both sexes show colour changes of the head during the breeding season;
- Mental or chin gland hypertrophy and function are described by Rose (1969), Winokur & Legler (1975) and Frye (1991a) in *Gopherus* spp. The mental gland is suggested to be a source of pheromones. The paired glands or tubercles are located on the ventrolateral aspect of the mandibles. Smaller but less developed glands are also present on females;
- Highfield (1996) describes further examples of species-specific sexual dimorphism.

Radiography

Though radiography is not a very effective method of determining gender, the presence of eggs demonstrated radiographically within chelonians confirms the sex to be female (Gibbons & Greene 1979; Holt 1979).

Ultrasonography

Kuchling (1989), Rostal *et al.* (1990), Penninck *et al.* (1991), Rübel *et al.* (1991a & b), Redrobe (1996) and Redrobe (1997) all describe ultrasonic examination of the female reproductive tract of mature and reproductively active females. At our surgery we have also found the coelomic testicles of male tortoises can be identified in mature specimens greater than 800 g where there is room to insert a probe in the inguinal fossa. Ultrasonography is discussed elsewhere in this book.

Hormonal sexing

Owens describes the determination of sex in immature sea turtle populations based upon testosterone levels. Fluid remaining in the egg at hatching can be used (Owens 1996). Changes in blood levels of testosterone in immature turtles have also been used to determine sex in sea turtles (Owens 1996). However, this method appears to have limitations, since some studies appear to have shown that testosterone levels in the male may decrease with time after capture. This may also give a false elevation in proposed females (Owens 1996).

Incubation conditions

Environmentally sex determined (ESD) species can potentially be sexed by temperature of incubation. Other factors such as oxygen tension may also be influential and as our understanding of the differences between species increases, the gender of a nest may be known with a high degree of accuracy. It has been suggested that oxygen concentrations during incubation affect sex ratio (Highfield 1996).

Endoscopy

Various workers, including Limpus *et al.* (1985), Schildger (1987), Kuchling (1989) and Divers (1997b), describe reproductive

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endoscopy in chelonians. This subject is also described in the endoscopy section of this book.

Intersexuality

Intersexuality has been observed in both wild (Limpus *et al.* 1982) and captive (Highfield 1996; Grazioli & Frye 2002) chelonians. Highfield (1996) suggests that it may be a result of incubation of ESD eggs at a constant temperature in the threshold region where both male and female offspring occur.

Both Pieau (1975) and Limpus *et al.* (1982) describe the persistence of the paramesonephric duct in phenotypic males and this is potentially the result of incubation at the threshold temperature zone as mentioned. Limpus *et al.* (1982) suggest that intersexuality may influence fertility rates in males.

Mating and hybridisation

In most terrestrial species the male mounts the female from behind and above. The male penis is then engorged and passes into the female cloaca. Aquatic species mate under water, anecdotally in localised breeding areas. Mating is often preceded by male courtship behaviour. This may include characteristic vocalisations and aggression. Males of some species repeatedly butt females with their gular scute. Many males will bite the head and limbs of cornered females. This behaviour, whilst appearing unpleasant and aggressive to an observer, may be necessary to make females submissive for mating. In combination with pheromones, and mating itself, it may also induce fertile ovulations in receptive females.

Male *Testudo hermanni* or *Geochelone pardalis*, with their spiked tail tips, can occasionally rake and tear the cloaca of those they mount. Larger species, such as *Geochelone sulcata*, corner and immobilise female tortoises by pinning them to the walls of any enclosure. Mating in these circumstances can be forceful.

Traumatic injuries are easily induced in those situations where male tortoises are confined in enclosures with other tortoises of either sex. Close observation is required to prevent shell, cloacal and dermal injuries. This author (SM) is regularly presented with both male and female tortoises suffering from extensive shell trauma and cloacal tears as a result of inappropriate housing with males. It is our practice to house isolated yet sexually active males with decoy walking boots as these provide them with something to mate and work on, sparing in-contacts from unnecessary trauma.

Highfield (1996) suggests that male tortoises show a definite preference for large females. In order to establish a successful breeding colony he suggests that all female tortoises be kept similarly sized. Larger or possibly infertile females are best removed, as they may result in male distraction.

As already suggested, male pheromones, courtship behaviour and mating may help induce ovulation and maintain normal female reproductive function. Highfield (1996) suggests that the violent advance of males towards females may stimulate egg production or receptivity, whilst DeNardo (1996) comments that some reptiles, including snakes, require the presence of a male to proceed beyond pre-vitellogenic follicular growth.

Sperm storage in mated females of some chelonian species may last four to six years (Frye 1991a; Galbraith 1993). This would suggest that only occasional mating is required to continue successful breeding in some wild populations. It would also suggest that not all ovulation is induced by mating. Multiple clutching and multiple paternities occur in some chelonians (Galbraith 1993). Artificial insemination may prove a valuable aid in the conservation of endangered species where mating is difficult in captivity. Control and assessment of ovulation may influence the success of such procedures.

Wood & Wood (1983) report hybridisation of *Chelonia mydas* and *Eretmochelys imbricata*. In Europe there are various anecdotal reports of hybridisation including *Geochelonia radiata* and *Geochelone carbonaria, Testudo marginata* and *T. ibera*, *T. ibera* and *T. graeca*, *T. hermanni* and *T. horsfieldi*. Highfield (1996) states that the external characteristics of the offspring in all instances of hybridisation he has observed have followed those of the sire. Nothing of the characteristics of the female species was obvious in the offspring of such matings. There are further reports emerging of hybridisation between various Asian species (Iverson *et al.* 2001) and various North American *Terrapene* spp. appear capable of hybridisation. Hybridisation should be eliminated from captive breeding programmes wherever possible.

Reproductive endocrinology

See **Endocrine system**, this chapter.

Folliculogenesis and vitellogenesis

Control of the female breeding season and the physiology of folliculogenesis in chelonians is poorly understood (Kuchling 1999). Although females of most chelonian species exhibit annual cycles, some breed only every three or four years (Carr & Carr 1970). The size and number of clutches per year varies considerably between species, sub-species and populations (Highfield 1996). Reptiles from temperate regions tend towards seasonal annual cycles, whereas those from tropical regions breed more continuously (Duval *et al.* 1982). It seems likely that natural endogenous cycles can be influenced by environmental factors. Environmental influences may affect both folliculogenesis and ovulation and factors stimulating and suppressing both probably differ.

Rainfall, moisture/humidity, food supply, social cues such as butting and other interactions with a suitable partner, photoperiod and factors within photoperiod such as light intensity, day length and the rate of change of day length may all have an influence on reproductive physiology. Such factors are known to influence avian reproductive cycles (Millam 1997). The follicular cycles of many chelonians, such as *Testudo* spp., may be further complicated by the possibility that induced ovulation may occur (Table 3.6).

Licht (1984) and Duval *et al.* (1982) describe two classes of chelonian vitellogenesis:

- In the first class, vitellogenesis and follicular growth typically begin in late summer or autumn and are completed just prior to winter hibernation;
- In the second type a modified form occurs in non-hibernating tropical reptiles, where slow follicular growth occurs continuously but is not completed until just prior to ovulation in the spring. Follicular development occurs directly before ovulation. The second type is less common.

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Table 3.6 Summary of endogenous and exogenous factors affecting vitellogenesis and ovulation.

Endogenous factors	Hormonal factors such as the balance of progesterone, testosterone and oestrogen (references as above) and/or thyroid hormones (as in avian species: Millam 1997).
Exogenous factors	• Light intensity. • Day length and its rate of change (Bartholomew 1959; Peaker 1969). • Ambient temperature and its rate of change (Peaker 1969; Licht 1972; Licht 1984; Duval *et al.* 1982; Bentley 1998). • Social cues, such as the presence of a suitable partner, the influence of pheromones (such as those released from male mental glands), butting, mating or other interactions (Rose 1969; Winokur & Legler 1975; Kuchling & Razandrimamilafiniarivo 1999). • Rainfall, moisture and humidity, nutrition and food supply (Licht 1984; Duval et al. 1982).

Some authors report situations where one chelonian species can alter its follicular development towards either of the cycles described. According to Moll (1979), *Chrysemys picta* is capable of showing both patterns of follicular development. In northern groups, vitellogenesis is almost completed before winter torpor, whereas southern populations complete follicular growth in spring, shortly before ovulation. Limpus (1971) described the nesting of *Chelonia depressa* in tropical northern Australia as occurring throughout the year, whereas in temperate southern Australia the same species nested annually, only in spring.

It is unclear which, if any, exogenous factors dominate the control of female reproduction. Licht (1972 & 1984) suggests that temperature is the most important stimulus for breeding in most reptiles. But it is not clearly established that temperature is the dominant factor in any chelonian species. Bentley (1998) supports the suggestion that light is of less importance than temperature in regulating the female reproductive cycles of poikilotherms, but Vivien-Roels *et al.* (1979) produced convincing evidence of circannual and circadian fluctuations in the serotonin and melatonin of wild *Testudo hermanni*. Both the maximum concentration and the amplitude of circadian fluctuations of these chemicals were increased during the breeding season. Bentley (1998) reports that photoperiodic effects are described in reptiles, but are rare. It seems plausible that both heat and light cycles may influence folliculogenesis and that neither has absolute control.

Ovulation

Ovulation may be induced, in some way, by the presence of a male. This could involve pheromones, male courtship behaviour such as butting and biting, or the act of mating itself (McArthur 2000a). Kuchling & Razandrimamilafiniarivo (1999) give strong evidence supporting the concept that some chelonians are induced ovulators. Regular socialisation of females with an appropriate male might reduce the prevalence of follicular stasis described later. Backues & Ramsay (1994) propose a similar hypothesis in oviparous lizards. Galbraith (1993) implies that some species of chelonians retain the ability to ovulate some time after a successful encounter with a male. It is plausible that mature isolated female chelonians might revert to a physiological state requiring induced ovulation, but, following successful contact with a male, may experience spontaneous fertile ovulations for several years once more.

Fertilisation and egg development

Frye (1981 & 1991a) comments that isolated female tortoises are temporarily capable of egg laying in the absence of a male tortoise, particularly in the first year of captivity. In such cases the eggs may be viable and worthy of incubation, provided that the female has been exposed to a male tortoise in the immediate preceding years. Where a chelonian has been isolated for more than four years, fertile ovulations are unlikely.

Galbraith (1993), Kuchling (1999a & b), and Kuchling & Razandrimamilafiniarivo (1999) give evidence for sperm storage and multiple paternity in chelonians. Multiple paternity is considered to decrease the relatedness of offspring and to increase sperm competition. This reduces the chance of future inbreeding. Sperm storage means that only occasional mating is required to continue successful breeding in some wild habitats. Galbraith (1993) reports the site of sperm storage within mated females to be the narrow tubules of the albumin-secreting region of the oviducts. Gist & Congdon (1998) suggest that sperm stored in the oviducts of *Sternotherus odoratus* and *Trachemys scripta* was likely to be used in the fertilisation of eggs ovulated in the second and subsequent clutches. According to Frye (1991a), internal fertilisation occurs within the oviducts, presumably after ovulation has been induced either by mating or, in the case of stored sperm, by external factors such as food availability or temperature.

As ova are fertilised and continue down paired oviducts, yolk and shell are added. Frye (1991a) describes the cranial and middle oviduct to have a mixed yolk/shell producing function and the caudal oviduct to be a shell-gland region where mainly calcified shell is produced. Radiographic and ultrasonographic studies of gravid cases presented at our surgery, showing early calcification in the period between mating and oviposition, support this suggestion. This period appears to be under progesterone dominance as the result of secretion from the corpora lutea, as described later. Ultrasonic and radiographic examination of the female reproductive tract are described in later sections of this book.

Oviposition

Highfield (1996) summarises pre-nesting behaviour in captive terrestrial chelonians as reduction in food intake, territorial behaviour, climbing and 'perimeter walking'. He points out that some behaviour patterns are species specific.

Oviposition in terrestrial females such as *Testudo graeca* appears to involve a nesting site chosen on the basis of ground

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temperature. Highfield (1996) recorded a ground temperature of 38°C–41°C as preferential in this species. Female chelonians in the wild may adopt a territory that has a suitable south-facing slope where exposure to the sun is likely to incubate eggs appropriately and burrowing is possible. This can rarely be provided to captive chelonians. Failure to provide a suitable nest site, with a suitable substrate at a suitable temperature is a major cause of dystocia in captive breeding females (Lloyd 1990). During oviposition, most chelonians excavate a nest chamber with their hind limbs and this may take several hours. Boyer & Boyer (1992) suggest a nesting area for a semi-aquatic turtle should have substrate to a depth equal to twice the length of the carapace, and an area four to five times that of the carapace, it being preferable to offer even more.

Oviposition in many species of marine turtles, including *Chelonia mydas*, is reported to involve an astounding feat of navigation: the female is assumed to return to the beach from which she entered the sea as a hatchling. This has the complication that the beach may have changed considerably in the 20 or so years since she hatched, particularly with the increase in tourism we have recently experienced. The ability of females to relocate to secondary sites may be essential to the future survival of turtle populations. An example of this problem exists in Cyprus. Here, an egg relocation project for *Chelonia mydas* recovers eggs from nests that cannot be protected from predation or from human nocturnal activity. It is too early to know if turtles that result from relocated hatchlings will return to the Nature Reserve beach to which they were moved (Demetropoulos & Hadjichristophorou 1995). Field reports suggest that relocation of nesting females previously bonded to areas of southern Cyprus may have occurred, with female landings shifting to sparsely-occupied northern beaches as a result of intense commercial development of beaches in the south of the island (Godley: personal communication).

Vibrio mimicus contamination of the sand increased significantly during the arrival of Olive Ridley sea turtles (*Lepidochelys olivacea*) at Ostional Beach, Costa Rica (Acuna *et al.* 1999). Contamination of eggs was proposed by contact with the sand. *Vibrio mimicus* was isolated from all nests tested. This evidence would support efforts of conservationists trying to reduce human consumption of turtle eggs. It may also support moves to relocate sand hatcheries at turtle stations on an annual basis as opposed to reusing the same site for several years.

Gravid Mediterranean female *Chelonia mydas* observed by this author (SM) will generally come ashore in the evening or at night. A gravid female may dig several nests over a period of several hours before satisfying herself with a nest and finally releasing her eggs. If disturbed before she has committed herself to laying, she is likely to return to the sea and jettison her eggs. If interacted with once egg laying has commenced, she appears transfixed and unaware of her company. Knowledge of this has allowed photography-based ecotourism to evolve. Photographs of laying females may be taken once oviposition has commenced, but such practices must be viewed with caution, as they may be remembered, and deter females from making future return visits or laying multiple clutches in that season. There is anecdotal evidence from various turtle stations visited by this author (SM) that many gravid females do indeed return despite camera flashes. It is essential that those beaches where marine turtles return to lay eggs be maintained in an appropriate manner. Airport lights, lights in hotels and bars, fires and beach parties may all prevent gravid females landing or distract hatchlings during their descent from the nest to the sea. Some workers suggest disturbed females may be forced to abandon their nesting attempt, and may not advance up the beach in future attempts, making the nest susceptible to flooding if it lies too near the water table.

The stability of marine turtle populations hangs in the balance and is dependent upon habitat preservation within countries where hotel complexes and beach bars are major sources of income and nature reserves struggle for popularity. It is hard to explain to those on low incomes why they should not profit from the commercial development of local beaches. Unfortunately, the commercialisation of beaches suited to marine turtle nesting, and consequent habitat destruction, could result in a serious decline in the global turtle population. It may be necessary to incorporate ecotourism in nest protection schemes in order to satisfy both humans and turtles (Demetropoulos & Hadjichristophorou 1995; Vieitas *et al.* 1999).

Clutch size

Clutch size is species specific, with marine turtles such as *Chelonia mydas* capable of laying in excess of a hundred eggs several times in a single season, and some terrestrial species such as *Malacochersus tornieri* possibly laying just one egg a season (Darlington & Davis 1990; Highfield 1996). However, the number of eggs laid by any given species is not necessarily consistent. In contrast to the comments above, *Malacochersus tornieri* was found to lay one egg per clutch, in up to six clutches per year, by Schmalz & Stein (1994). In some captive colonies, *Geoemyda spengleri* and *Rhinoclemmys* spp. typically lay one egg, though occasionally two (Innis: personal communication). In reference texts, however, it is suggested that *Rhinoclemmys pulcherrima* species typically lay up to three eggs (Pritchard 1979: Pauler 1990).

Most Mediterranean tortoises have a typical clutch size of four to ten eggs, but this varies considerably between species and sub-species. *Testudo hermanni hermanni* (the western population of Hermann's tortoise, inhabiting France and Italy) have a typical clutch size of only three eggs, for example, whilst the eastern subspecies *T. h. boektgeri* typically lays clutches of six to ten eggs. Egg sizes also vary greatly between various species and subspecies (Highfield 1996).

Multiple clutches are possible within each breeding season. In the case of some species, including marine turtles, this is assumed to occur without further mating as a result of sperm storage (Galbraith 1993).

Egg management

The anatomic structure and the biochemical composition of tortoise eggs are described in depth by Smith (1984). Sahoo *et al.* (1998) suggested that the yolk-albumen of Olive Ridley turtle eggs contained elements (other than calcium) sufficient to achieve normal embryonic development with appropriate respiration, and that the shell of the egg provided 60% of the calcium requirements, with absorption from the shell occurring from day 40 of incubation onwards.

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Eggs produced naturally, or by medical induction or surgical removal from an egg-bound turtle, may be fertile, especially if produced in the presence of a compatible male. Eggs laid naturally may be fertile if the female has been maintained in the company of a compatible male tortoise, either at the time or within recent years.

The use of an incubator should generally be encouraged wherever veterinarians encounter potentially fertile non-hybridised eggs, e.g. following induction of retained eggs. A suitable egg container may be created from a small plastic food box containing substrate such as sand, soil or vermiculite. The viability of such eggs should never be guaranteed.

Several commercial incubators suited to chelonians are now available. Two types of self-construct incubator are easy for keepers to arrange:

- A box can be heated using a thermostatically-controlled heat pad. Eggs can be placed in a container lying within this box, which is usually lined with soil, sand or vermiculite as substrate. Misting and moistened containers of gravel can be used to increase humidity;
- A fish tank (about 70 cm by 30 cm) can be half-filled with water and maintained at a stable suitable temperature using a heating element. Here the eggs can be placed into a floating container. Alternatively the container can be placed upon a stone or brick within the water so that the lower part of the container stands in the water. The container within the incubator is probably best covered, as condensing water may drip from the incubator lid and suffocate the developing embryo (Fig. 3.35).

In both cases humidity must be maintained in a manner appropriate to the species. Further details of incubation can be found in Highfield (1996).

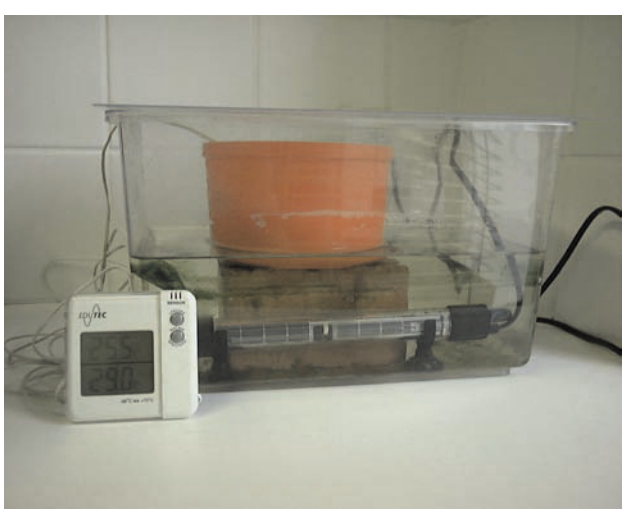

Fig. 3.35 Simple and efficient low-budget incubator for tortoise eggs. This example has been created using a small plexi tank and an aquarium heating source. Eggs are imbedded in dry sand in the bowl in the middle. The bowl is placed on a brick with its lower part standing in the water. The top of the bowl should be covered with a second, thin, slightly-perforated plexi plate, in order to avoid dripping of condensed water from the tank lid onto the eggs. The heat is controlled with a simple electronic temperature probe.
(Courtesy of Jean Meyer)

Environmental sex determination (ESD)

Incubation temperatures play a significant role in determining the sex of the hatchlings of many chelonian species, including soft-shell turtles such as *Trionyx spiniferus*, snapping turtles (Yntema 1976), and sea turtles (Yntema & Mrosovsky 1982; Standora & Spotila 1985; Desvages *et al.* 1993). Semi-aquatic turtles such as *Emys orbicularis* (Pieau 1975; Pieau & Dorizzi 1981; Pieau 1982) and terrestrial species such as *Testudo graeca* (Pieau 1975) are similarly affected, as are further species such as *Geochelone carbonaria* and *Testudo hermanni*, which are described by Highfield (1996). Some chelonian species such as *Clemmys insculpta* and *Chelodina longicollis* appear to have genetically-determined sex.

According to Madge (1994):

- Eggs of the spur-thighed tortoise (*Testudo graeca*) produced males at 29.5°C and females at 31.5°C. Both sexes were produced in the threshold region 30°C–31°C.
- Eggs of the European pond turtle (*Emys orbicularis*) produced males at 27.5°C and females at 29.5°C. Both sexes were produced at 28°C–29°C.
- Eggs of the loggerhead turtle (*Caretta caretta*), kept at 28°C or below, all developed into males, while those kept at 30°C or above all developed into females. At 29°C both males and females resulted.
- Map terrapins (*Graptemys* spp.), the slider terrapin (*Trachemys scripta*) and the painted terrapin (*Chrysemys picta*) all produced mostly males at 28°C and mostly females at 30°C. Both sexes were produced at 29°C.
- In contrast, for the snapping turtle (*Chelydra serpentina*), both extremes, above 30°C and at 20°C, produced mainly females, while intermediate temperatures of 22°C–28°C produced mainly males.
- Sexual differentiation in the soft-shelled turtle (*Trionyx spiniferus*) appeared to be independent of temperature.

The sexual differentiation of the following species of sea turtle embryos appears primarily to be determined by temperature:

- *Caretta caretta* (Yntema & Mrosovsky 1979; Mrosovsky 1980; Mrosovsky & Yntema 1980; Limpus *et al.* 1985);
- *Chelonia mydas* (Miller & Limpus 1981; Yntema & Mrosovsky 1982; Morreale *et al.* 1982);
- *Lepidochelys olivacea* (Ruiz *et al.* 1981);
- *Dermochelys coriacea* (Rimblot *et al.* 1985);
- *Eretmochelys imbricata* (Mrosovsky *et al.* 1992).

Highfield (1996) gives the following as a guide for the incubation of *Testudo hermanni* (Table 3.7):

Table 3.7 Effects of temperature in incubating *Testudo hermanni* eggs (Highfield 1996).

Temperature	Effect
<26°C	Eggs usually die
26–29.5°C	All male offspring, 74–140 days
30–31.5°C	Mixed offspring
32–34°C	All female offspring, 60–75 days
>34°C	Deformed hatchlings

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It is proposed that when eggs are incubated at higher temperatures, in the leatherback turtle (*Dermochelys coriacea*), the enzyme aromatase is activated and this in turn converts available androgen steroid substrate to oestrogens. Without aromatase it is proposed that no oestrogen will be produced and the embryo will remain male (Desvages *et al.* 1990; Gross *et al.* 1995; Owens 1996).

Normal development and anatomy

The amniotic egg of reptiles represents a major evolutionary landmark, allowing independence from the aquatic environment required by fish and amphibians for reproduction. It is an enclosed, but permeable structure, providing an aqueous environment and nutrition for the developing embryo. Eggs are generally deposited in a protected environment and left to develop (with few exceptions) without maternal care. Selection of a nest site with proper temperature, humidity, and gas permeability is therefore of utmost importance.

The shell of reptilian eggs generally consists of two layers: the external mineral layer, and the internal fibrous layer, or shell membrane (Ewert 1985). The mineral layer varies from sparse aggregates of mineral in the flexible eggs of some turtles, to a thick, rigid layer in the brittle eggs of many turtles and tortoises (Ewert 1985). This variation has important consequences for the water relations of the embryo as discussed below.

Soon after oviposition, the embryo begins to rise to the dorsal part of the egg as the yolk settles ventrally. It will remain in this basic orientation throughout development. Thus, at hatching, the embryo should be found resting dorsal or slightly lateral to its yolk remnant (Ewert 1985).

As development proceeds, the extra-embryonic membranes begin to form. The first to form is the *vitelline*, or yolk-sac membrane, which becomes vascularised, providing the nutritional and early respiratory needs of the embryo. Later, the *amnion*, *chorion*, and *allantois* form. The innermost membrane, the amnion, surrounds the embryo, providing fluid support. The allantois provides a receptacle for nitrogenous waste, and, by mid-development, fuses with the outermost membrane, the chorion, which functions as the major gas exchange organ for the embryo.

Air spaces, such as those found in bird eggs, form variably in reptile eggs. They seem to occur most commonly in rigid-shelled turtle and crocodile eggs (Ewert 1985; Ferguson 1985). The location of the air cell is variable, sometimes being in the albumen, between layers of the shell membrane, or between the shell membrane and the mineral layer (Ewert 1985; Ferguson 1985; Packard 1982). The importance of the air cell is unclear, but it may be important for early pulmonary respiration prior to *pipping* (Ferguson 1985).

Hatching is a complex process, and possible stimuli for hatching are discussed below. The full-term embryo, utilising the egg tooth, or *caruncle*, and movement of the limbs, 'pips' the egg and begins pulmonary respiration. The remaining yolk and blood in the extra-embryonic circulation is absorbed by the embryo. After a variable period of time, sometimes several days, the neonate will leave its egg.

Some incubation advice is given by Innis (1995), Highfield (1996) and Lutz & Musick (1996). Species-specific advice is becoming increasingly available and this should be sought when establishing breeding programmes. The effects of alterations in temperature provision vary with species. Requirements for incubation humidity, oxygen concentrations and other conditions are at present poorly recorded. Highfield (1996) suggests that oxygen levels during egg incubation may also influence the sexual differentiation of embryos within eggs. This hypothesis is supported by the differences in oxygen and carbon dioxide concentrations around sea turtle nests during incubation described by Ackerman (1996).

Eggs can be candled using a bright light to see if the yolk has settled, and to monitor the development of embryonic vasculature, but this is not 100% reliable as an indication of fertility (Raiti 1995a, Highfield 1996). Infertile eggs tend to dry out, and embryonic death is suggested by lack of weight. Ultrasonography can also be used to visualise the content of eggs and may assist in viability assessment.

Egg chamber structure, temperature and oxygen gradient

Work on the methods with which sex is determined during incubation reveals important aspects of egg-chamber structure and environment that affect the annual population and sex ratio of the wild population. Most chelonians excavate a nest with their hind limbs. During oviposition, eggs are usually laid in a cylinder-shaped egg chamber, which is then covered over with soil or sand.

A cylindrical shape causes eggs at the top to be incubated at a warmer temperature. This may mean that the top eggs hatch earlier than those at the bottom. It may also mean that a balance of sexes is produced within the nest. If the overall nest temperature does cause a specific sex and incubation period to occur then the changes in beach temperature over the season will affect the sex ratio of the hatchlings.

Ackerman (1996) describes changes in oxygen and carbon dioxide concentration in the egg chamber. It is possible that changes in these parameters, or in humidity, alter the sex of hatchlings locally within a nest and complement the influence of temperature described above.

INFERTILITY AND EMBRYONIC DEATH

One of the most frustrating problems encountered by breeders of oviparous reptiles is the death of grossly normal, full-term embryos prior to hatching (Innis 1995) (Figs 3.36–3.42). Entire clutches or only a percentage of fertile eggs may be affected (Plummer 1994; Ewert 1985). Late embryonic death is a common problem observed in nearly all reptile taxa. Unfortunately, diagnostic testing of dead embryos is infrequently performed, and detailed records of parental nutrition, age, incubation parameters, etc. are often not recorded.

Temperature, humidity, substrate type, substrate saturation and gas concentrations in the incubator all affect development. These factors are all somewhat interdependent.

Temperature

It is clear that incubation temperature is an important factor regulating the rate of embryonic development, oxygen consumption

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Fig. 3.36 Late embryonic death may affect just one or two eggs within a clutch or all of them.

Fig. 3.37 In many cases, embryos affected by late embryonic death appear normal.

Fig. 3.38 Size difference between the hatchling with the removed yolk sac Figs 3.40–3.42 (on the right), and a clutch mate both aged six months.

As the hatchlings had enjoyed identical care and nutrition, it would appear likely that loss of yolk sac nutrition has resulted in decreased growth and development during early life.
(Courtesy of Jean Meyer)

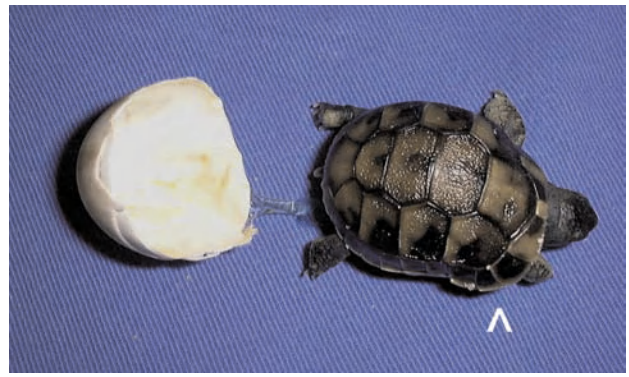

Fig. 3.39 Carapacial deformation of a hatchling (*T. hermanni*) which was stuck for two days in its half-opened shell. This deformity resolved within 48 hours after complete hatching.
(Courtesy of Jean Meyer)

Fig. 3.40 Retained yolk sac (*T. hermanni*).
(Courtesy of Jean Meyer)

Fig. 3.41 The hatchling illustrated in Fig. 3.40 was replaced in its shell for about five days. During this time the yolk sac diminished by half. The yolk sac remnants subsequently required ligation and removal, as they were inadvertently torn open by the hatchling.
(Courtesy of Jean Meyer)

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Fig. 3.42 The plastron of the same hatchling as in Figs 3.38 and 3.41 a week after yolk-sac removal. The umbilical slit is closed. (Courtesy of Jean Meyer)

and incubation length (Deeming & Ferguson 1991; Kam & Lillywhite 1994). For example, for the smooth soft-shell turtle (*Apalone mutica*), the average incubation length at 27°C is 75 days, while at 33°C the average length is 50 days (Plummer 1994).

For most species, there is a range of temperatures within which development can proceed normally. Abnormal incubation temperatures may produce major congenital defects such as visceral herniation, spinal defects, anophthalmia, etc.; however, minor defects such as supernumerary scutes may also be seen (Deeming & Ferguson 1991; Ross & Marzec 1990). Occasionally, however, inappropriate incubation temperatures may result in grossly normal embryos that die late in development. For example, 21 of 33 *A. mutica* embryos incubated at the relatively low temperature of 24°C died late in development without gross deformity (Plummer 1994).

The effect of temperature fluctuation on development depends on the magnitude and temporal characteristics of the fluctuation. Large, rapid temperature fluctuations may pose significant physiological stress for the embryo (Deeming & Ferguson 1991) and have the potential to cause death. On the contrary, gradual temperature fluctuations may be crucial to successful hatching of some species, as discussed below.

Humidity and substrate saturation

Depending on the thickness of the eggshell mineral layer, incubator humidity and substrate hydration may significantly affect embryonic development. Some rigid-shelled eggs, such as those of the spur-thighed tortoise (*Testudo graeca*), are relatively impermeable to water and show little variation in hatchability at high, medium or low incubation humidity (Highfield 1990b). Flexible eggs, however, are more permeable and under favourable conditions will take up water during incubation. If water availability is limited, embryonic dehydration and death may occur. For example painted turtle (*Chrysemys picta*) eggs are quite sensitive to dry conditions, suffering high mortality (Innis: personal observation).

Incubator humidity and substrate saturation are often thought of as synonymous, while they are actually separate entities. While it is true that very damp substrates tend to produce high humidity as evaporation occurs, it is possible to have incubator humidity levels of 90–100% while the substrate is quite dry. This is an important point, as the water requirements of the embryo must be balanced with the effect of high humidity and substrate saturation on gas exchange, as discussed below.

There is some evidence that fluctuation of humidity or substrate saturation may be important for hatching. Highfield (1990) observed that eggs of several tortoise species seem to be stimulated to hatch by sharp increases in incubator humidity, postulating that reduction of gas exchange and a rise in carbon dioxide stimulates hatching behaviour. If such hydric fluctuations are ignored under artificial incubation, hatching could be delayed long enough to cause death.

Gas exchange

Oxygen consumption and carbon dioxide production by reptile embryos increase dramatically during development, with oxygen consumption showing a sigmoidal or exponential increase over time (Deeming & Ferguson 1991). As a result, oxygen concentrations in the incubation environment can be expected to fall, as carbon dioxide concentrations rise, throughout incubation. Lutz & Cooper (1984) demonstrated that, in American crocodile (*Crocodylus acutus*) nests, oxygen concentrations may fall from 20% at laying to 17% at hatching while carbon dioxide concentrations rise from 0.6% at laying to 2% at hatching. For poultry eggs, hatchability is greatly reduced when oxygen concentrations fall below 20% and carbon dioxide concentrations rise above 0.5% (Romanoff 1972; North 1984).

Hypoxia and hypercapnia should be considered as possible causes of late embryonic death, particularly under artificial incubation conditions. Ackerman (1981) showed that embryos of loggerhead (*Caretta caretta*) and green sea turtles (*Chelonia mydas*) show reduced growth rates and lower hatching success when oxygen availability is limited. Numerous anecdotal reports support this concept, indicating that incubation in sealed, infrequently-opened chambers results in high numbers of fully-formed, dead-in-shell embryos.

It is important to realise that substrate saturation, incubation temperature and humidity also affect gas exchange of the egg. Kam & Lillywhite (1994) showed that Florida red-bellied turtle (*Trachemys nelsoni*) embryos incubated at 32°C consume more oxygen and are more sensitive to experimental hypoxia than embryos incubated at 27°C. It is likely, therefore, that eggs incubated at the high end of the 'normal' temperature range will be more likely to suffer from hypoxia than eggs incubated at more moderate temperatures.

The effect of substrate moisture content and incubation humidity on gas exchange has only been studied in several species. *In vitro*, American crocodile (*Crocodylus acutus*) eggshells are ten times more permeable to oxygen at 70% humidity than at 100% humidity, presumably due to partial saturation of the eggshell with water (Lutz 1980). The *in vivo* significance of this observation is not clear, however, since the reduced permeability at higher humidity may not be significant enough to reduce oxygen diffusion below the lethal threshold. Kam & Lillywhite (1994) found that even in hypoxic conditions, oxygen consumption of Florida red-bellied turtle (*T. nelsoni*) embryos was identical in

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two different hydric environments. The two environments used, however (substrate water potentials of 4 kPa and 13 kPa), can both be considered to be 'wet' environments, and one cannot conclude from this data that oxygen consumption of this species is the same under 'wet' vs. 'dry' conditions.

As a generality, it is likely that there are various upper limits of eggshell saturation that can be withstood by various species before hypoxia leads to death. As an extreme example of the effect of saturation, it is known that 12-hour submergence of 30-day or older American alligator (*Alligator mississippiensis*) eggs causes embryonic death (Ferguson 1985). In less extreme conditions, very wet substrates are likely to impede gaseous diffusion through the eggshell and also lead to eggshell saturation. This effect must be avoided while still providing adequate hydration for normal growth of the embryo.

Maternal nutrition

It has been known for many years that eggs laid by nutritionally deficient chickens suffer high mortality. For example, hens fed a diet slightly deficient in riboflavin lay eggs that show high mortality one to two days prior to hatching (Romanoff 1972). Similar effects are seen with deficiencies of biotin, folic acid, vitamin A, etc. (Romanoff 1972). There is no reason to doubt that such deficiencies may also affect reptile embryos. In a group of crocodile eggs, 15% of captive-produced eggs showed late embryonic death, while eggs collected from wild females, incubated under the same conditions showed very few such deaths. While other explanations are possible, a nutritional deficiency of the captive females is hypothesised (Ferguson 1985).

The calcium metabolism of captive reptiles has received a great deal of attention and is likely to be of particular importance for normal egg production. Packard (1992) demonstrated that the calcium for embryonic growth of green iguanas (*Iguana iguana*) is derived from both the eggshell and yolk. Given this, it is clear that adequate calcium must be available during vitellogenesis and shell deposition to meet the embryos' later needs.

Until we learn more about specific nutrient requirements for individual species of reptiles, late embryonic death due to maternal nutritional deficiency should be considered to be quite likely.

Substrate effects

In certain species, the composition of the substrate in which eggs are deposited is important for successful hatching. The best-described example of this effect occurs in crocodilians. During development, erosion of the mineral layer of the eggshell occurs, weakening the shell to facilitate hatching. It is unclear whether this phenomenon is a result of bacterial or fungal degradation, or due to the action of carbonic acid formed by expired carbon dioxide under conditions of high humidity (Ferguson 1985). The carbonic acid theory is given some support by the observation that crocodile eggs can be successfully hatched without incubation medium as long as humidity is above 90% (Ferguson 1985).

Although at this time, degradation of the mineral layer is not thought to be of importance for hatching in other reptile taxa, it should be considered where other causes of death can be ruled out. In an attempt to decrease late embryonic death of Burmese brown tortoises (*Manouria emys*), one breeder attempted to use an incubation substrate composed of a mixture of several types of organic matter to promote eggshell degradation. The attempt was unsuccessful, and the cause of death remained unclear (Love 1994).

Egg position, rotation and vibration

Unlike avian eggs, reptile eggs are not naturally turned during incubation. Lacking the chalazae of avian eggs, reptile eggs are more sensitive to physical movement. Most discussions of reptile egg incubation have addressed this concern by recommending that the egg be maintained in the same position in which it was laid when transferring it to the incubator. Under scrutiny, however this recommendation may only be partially correct. Immediately after laying, the egg contents and embryo have not become fixed in their later orientation, thus it is likely that repositioning will not affect later developments. Later in development, however, rotation is likely to reduce hatchability, as demonstrated by Ewert (1979), who showed that hatching success of alligator snapping turtle (*Macroclemys temmincki*) eggs rotated weekly was 63% compared with 81% success of non-rotated eggs. Mortality may be caused in two ways: during the act of rotation, shearing forces may tear the chorioallantoic circulation away from the shell membrane, or the weight of the yolk may come to rest on top of the developing embryo (Ewert 1979). Nicol (1991) reported late embryonic death of red-foot tortoise (*Geochelone carbonaria*) eggs that was attributed to the location of the incubator on top of a dresser, which was frequently opened, causing jarring of the eggs. Contrarily, Ewert (1979) reports successful development of turtle eggs in natural nests close to railroad tracks where the ground noticeably vibrated as trains passed. It is likely that some vibration is tolerated, but that excessive jarring may be lethal.

Infection

Embryonic death due to infection by bacteria, fungi or viruses is known to occur in avian species (Olsen *et al.* 1990). One should expect that similar infections can affect reptile eggs. Infection may theoretically arise from salpingitis, cloacal contamination or environmental contamination. Obviously, natural nest sites are not sterile, and healthy eggs possess some ability to resist infection, but this does not discredit the possibility of specific pathogens causing embryonic death. Further research in this area is needed, as no specific pathogens are considered to routinely cause embryonic death in reptiles at this time.

Genetic factors and inbreeding

It is known that inbreeding of chickens leads to increased embryonic mortality, particularly late in incubation (Romanoff 1949). However, many of these embryos show gross morphologic deformities. While gross deformities are likely with genetic incompatibility, late embryonic death of grossly normal embryos could also occur. For example, it is known that members of the same species from different geographic origins may have different incubation requirements (Ewert 1985). Thus eggs produced by pairing specimens from different geographic origins may have dissimilar

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incubation requirements. While some eggs may hatch in particular conditions, other eggs in the same conditions may fail to hatch.

It has been shown in poultry that the effect of nutritional deficiency on embryonic mortality for some strains of hens is greater than for others (Romanoff 1972). If this is true for reptiles, some females may have higher requirements for specific nutrients than other females of the same species. This could produce embryonic death due to genetically influenced nutritional deficiency.

Iatrogenic death

Premature manual pipping of reptile eggs can cause death of the embryo. In general, manual pipping of chelonian eggs is not recommended, particularly where normal incubation length is unknown. It is well documented that, for some tortoise species, individual eggs from the same clutch may hatch over a period of several days or weeks (Stearns 1985). Therefore, manually opening unpipped eggs, just because other eggs in the clutch have pipped, may prove fatal if the embryo is not yet prepared to hatch. Contrarily, there are probably circumstances when a weak embryo could be saved by manual pipping. Diagnostic techniques such as ultrasound, Doppler blood flow monitoring, or pulse oximetry should be evaluated for their ability to track reptile embryo development. With time, it is possible that these techniques could be used to determine when manual pipping may be of benefit.

Miscellaneous potential causes of embryonic death

An occasional embryo can be expected to die due to a random congenital deformity, but this occurrence should be rare. The effects of toxins or radiation on embryos have been studied in poultry chicks (Romanoff 1972), and should be considered as differential diagnoses for late embryonic death in reptiles. The role of antibiotic treatment of a female during vitellogenesis has not been studied. Nor has the possibility of paternal nutritional deficiency been addressed. It is possible that the age of the female may affect the quality of the egg, and reduce hatchability. Some evidence for this is seen in crocodiles, where old females produce more malformed hatchlings than middle-aged females (Ferguson 1985).

Diagnostic approach to embryonic death

An attempt should be made in all cases of embryonic death to determine its cause. The work-up is similar to that for avian embryos as described by Langenberg (1989). A complete history of parental husbandry, nutrition, medical record, geographic origin, breeding dates, incubation conditions, etc. should be obtained, and analysed for any potentially significant information.

Gross necropsy of the egg should be performed for all dead embryos (NB: these may explode when incised!). Candling the egg prior to opening will allow assessment of embryonic position and direct the site of the initial incision. Any lesions noted should be compared to the existing avian literature to attempt to determine an underlying aetiology,

Tissue samples of the embryo as well as of its extra-embryonic membranes should be obtained for histopathology. Unfortunately, the conditions of incubation favour rapid autolysis and results may be inconclusive. However, only by attempting to perform histology will we begin to recognise normal and abnormal embryonic anatomy. To date, histological evaluation of reptile embryos has been performed mainly by herpetologists studying embryonic development. There is a need for veterinary pathologists to become familiar with this work so that their services may be available to practitioners. By comparing histological lesions of reptile embryos to lesions described in avian embryos, a cause of death may be determined in some cases. For example, certain cardiovascular defects may be seen in chicken embryos that die due to hypoxia (Jaffe 1974).

Microbiological evaluation of the embryo is warranted, although results must be interpreted with caution and in combination with histological evidence of pre-mortem infection, since normal flora of reptiles may be partially established prior to hatching. For example, Ross & Marzec (1984) cultured *Pseudomonas* spp. from the oropharynx of a normal, healthy, Burmese python (*Python molurus bivittatus*), after aseptically removing it from its egg *prior to* pipping, on the expected date of hatching. Furthermore, contamination of egg contents *after* death can occur rapidly in the warm, humid conditions of incubation. Thus it is clear that simply obtaining a positive culture does not confirm death due to infection.

Toxicological analysis of the embryo may be considered if the possibility of toxin exposure exists.

Prevention of late embryonic death

As much information as possible regarding the natural nest conditions of the species of interest should be obtained. Invaluable information is gained from others who have successfully hatched a species, and an attempt should be made to mimic previously successful conditions as closely as possible. Incubation parameters should be recorded for the benefit of others, using accurate instrumentation to measure temperature, humidity and where possible, substrate water potential and incubator gas concentrations.

Ventilation should be adequate to prevent hypoxia, particularly for very large clutches of eggs. One system used by this author to maintain ventilation as well as humidity involves the use of an aquarium air pump to pump fresh air into the incubator. The tubing from the pump is placed within a container of water in the incubator, thus humidifying and warming the air at the same time. Although some species may tolerate extremely saturated substrates, poor gas exchange under such conditions could adversely affect other species. In these cases it would be better to use drier substrates with high ambient humidity.

The heat mechanism of the incubator should provide even heat output to all eggs. One commercial incubator used by this author was found to have a temperature variation of 5°C depending on the location of the egg, and produced an anophthalmic hatchling from the warmest location.

Maternal nutrition must be optimised, taking particular care to meet mineral, vitamin, and amino acid requirements. This can be difficult, as we have minimal understanding of the nutritional requirements of the different species at this time. Where possible, exposure to natural sunlight is encouraged. Females should be fed *ad libitum* during egg development. Breeding groups or pairs

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should consist of individuals of similar geographic origin whenever possible, but inbreeding should be avoided.

Opening 'overdue' eggs may be successful or disastrous. Certainly if prior experience shows very consistent incubation lengths, the decision to open unpipped eggs is simplified. Where the incubation length has been inconsistent, or is unknown, it is left up to the individual whether to pursue the aggressive or conservative route. Ross & Marzec (1990) provides an excellent description of manual pipping of python eggs.

Late embryonic death is likely to be a common problem for many reptile breeders. As our knowledge of natural history, nutrition, specific pathogens and egg metabolism grows, we may begin to minimise these mortalities. The herpetological community must be convinced of the need to make diagnoses and elucidate the aetiologies of this frustrating phenomenon.

Male infertility

True infertility must be differentiated from lack of copulatory behaviour and unsuccessful copulation. Absence of copulatory behaviour may be noted if the animal is not truly male (incorrect gender identification). If gender identification is correct, absence of copulatory behaviour may occur if the pair is genetically incompatible (e.g. different subspecies), if the male is ill or if proper environmental stimuli are lacking. Normal copulatory behaviour, without successful copulation, may occur due to rejection by the female, penile pathology or pathology of the male or female cloaca. A thorough physical examination should be performed to evaluate for cloacitis, cloacal foreign bodies, penile pathology, etc. (Innis & Boyer 2002a).

If true infertility (absence of sperm production) is suspected, a thorough review of husbandry and health is warranted. Sperm production in most chelonians occurs at the end of the prior reproductive season and sperm are stored until the following year (Kuchling 1982; Kuchling 1999c).

Some sea turtle species produce sperm immediately prior to the reproductive season (Wibbels *et al.* 1990). Annual cycles of sperm production are affected by hormonal changes in response to temperature and photoperiod fluctuation for temperate species, as well as rainfall patterns for tropical species. Testosterone levels are generally highest during spermatogenesis and some species also show a second testosterone peak during the breeding season (McPherson *et al.* 1982; Licht 1982; Kuchling 1999c).

Testosterone levels should be evaluated in cases of suspected infertility. Several studies have evaluated testosterone levels of free-ranging and captive chelonians, and may be used as a guide until normal values for more species are available (Licht *et al.* 1979; Licht 1982; Licht *et al.* 1985; Mendonca & Licht 1986; Wibbels *et al.* 1990; Rostal *et al.* 1994; Owens 1997; Rostal *et al.* 1998a; Rostal *et al.* 1998b; Kuchling 1999c).

To try to document sperm production it may be helpful to evaluate urine sediment of males, as sperm are sometimes visible. In addition, examining cloacal flush samples from both male and female after copulation may be helpful. Coelioscopic examination of the testes is easily performed, and endoscopic biopsy or needle aspirate of the testicle may be considered. Electroejaculation of chelonians has been described but has not been widely utilised (Platz *et al.* 1980; Wood *et al.* 1982; McKeown *et al.* 1982).

Objective parameters of semen quality of green sea turtles (*Chelonia mydas*) were established by Wood *et al.* (1982). Since sperm production may only occur seasonally, evaluation of the patient over a one- to two-year period may be needed to document true failure of spermatogenesis. Confirmed absence of spermatogenesis should prompt a thorough review of the health status and husbandry of the patient. In particular, diet, photoperiod, temperature, humidity, rainfall and habitat design should closely mimic that of the animal's natural environment.

ENDOCRINE SYSTEM

Pancreatic hormones

The location of the pancreas is variable but, as in mammals, it can usually be found adjacent to the proximal duodenum. In chelonians, and in vertebrates, its physiological functions are both exocrine and endocrine.

Two main pancreatic hormones, insulin and glucagon, are produced in the B- and A-cells of the islets of Langerhans, respectively.

Insulin

Insulin is an anabolic hormone, stimulating glucose uptake by the liver and the skeletal muscles. In *Trachemys scripta* and *Trachemys dorbigni*, its biochemical structure has an 86% homology in amino acid sequence to that of human insulin (Chevalier *et al.* 1996; Cascone *et al.* 1991). Despite chemical differences from the structure of the vertebrate insulins, due to conservation of essential biochemical sites, there is surprisingly little demonstrable variation in their specific biological activities. Pancreatectomy of turtles results in pronounced hyperglycaemia and glucosuria.

Simultaneously with postprandial glucose uptake, insulin also stimulates the uptake of amino acids by skeletal muscle and liver. In carnivorous, ectothermic vertebrates this effect of insulin on protein metabolism is more pronounced than in herbivorous species. However it is not yet clear if insulin stimulates amino acid transfer or if its primary effect is directed toward protein synthesis (Plisetskaya & Duguay 1993). The effect of insulin on lipid metabolism is to lower blood lipid concentration as a result of enhanced re-esterification of free fatty acids into triglycerides.

Glucagon

The hormone glucagon is secreted by pancreatic A-cells. Its effects are mainly glycogenolytic, gluconeogenic and lipolytic. This lipolytic effect couldn't however be demonstrated on isolated adipocytes in Hilaire's side-necked turtle *Phrynops hilarii*. (Da Silva & Migliorini 1990).

Somatostatin and pancreatic polypeptides (PP)

The pancreas also produces somatostatin and pancreatic polypeptides (PP). The biochemical structure of somatostatin has been isolated and sequenced for *Trachemys scripta* (Plisetskaya & Duguay 1993). Its metabolic effects are hyperglycaemia, depletion of liver glycogen content and elevation of plasma fatty-acid levels. These effects are to be further investigated in chelonians. The biological effects of PPs are similar in different vertebrates and include an increase in blood pressure and decrease in heart rate.

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Reproductive endocrinology

The pituitary gland, ovaries and testes all have roles in chelonian reproductive endocrinology (Bentley 1998; Kuchling 1999a & b). The physiological basis of sexual behaviour in male reptiles is poorly understood (Moore & Lindzey 1992). A photoperiodic effect has been reported to affect testicular activity in the red-eared slider (*Trachemys scripta*) by Burger (1937), however Duval *et al.* (1982) suggest that additional heat provided by increased lighting was a significant factor. By contrast, more is known of normal female reproductive endocrinology.

Progesterone

In reptiles, a distinct corpus luteum is formed following ovulation and available evidence suggests that it secretes progesterone (Bentley 1998; Kuchling 1999b). There is evidence that progesterone is one of the major steroids synthesised by the chelonian ovary (Klicka & Mahmoud 1977) and there is evidence that the corpus luteum of the chelonian is capable of synthesising progesterone (Klicka & Mahmoud 1972; Klicka & Mahmoud 1973; Chan & Callard 1974; Callard *et al.* 1976).

Progesterone was shown to inhibit ovulation completely, and reduce pituitary size, oviduct size and follicle size in the turtle *Chrysemys picta* (Klicka & Mahmoud 1977). Negative feedback inhibition of the release of a pituitary derived tropic hormone is suggested by Bentley (1998). Bentley further proposes that oestrogens and progesterone can influence the hypothalamic release of reptilian gonadotropins in a manner similar to mammals.

A potential physiological role for progesterone in the regulation of clutch size and maintenance of gravidity has been proposed (Klicka & Mahmoud 1977). In *Sceloporus cyanogenys*, Callard *et al.* (1972) propose that progesterone prevents follicular development both by direct action on the hypothalamus and, possibly, peripherally, resulting in inhibition of vitellogenesis.

Rostal *et al.* (1998) found that progesterone levels in the Galapagos tortoise (*Chelonoidis nigra*) displayed a sharp surge during the mating period that coincided with ovulation. However, Licht (1984) suggests that various chelonians, including the green sea turtle (*Chelonia mydas*), do not maintain post ovulatory levels of progesterone as expected and therefore the role of progesterone in chelonians may vary between species.

Oestrogen

In reptiles, oestrogen stimulates vitellogenesis, the production of lipophosphoproteins by the liver and their incorporation into the egg (Callard *et al.* 1972; Licht 1979; Duval *et al.* 1982; Kuchling 1999b). Ovarian maturation and follicular growth in the Galapagos tortoise (*Chelonoidis nigra*) coincides with elevations in oestradiol levels (Rostal *et al.* 1998) while ovarian maturation and follicular growth in *Dermochelys coriacea* follows an initial elevation in oestradiol levels (Rostal *et al.* 1996).

Testosterone

In *Chelonoidis nigra*, testosterone levels were elevated during the mating period immediately prior to ovulation. This rise was presumed to relate to receptivity of the female (Rostal *et al.* 1998). A similar rise in serum testosterone is observed in *Dermochelys coriacea* (Rostal *et al.* 1996) and *Lepidochelys kempii* (Rostal *et al.* 1997). However, both these species demonstrate unique elements of nesting behaviour, such as *arribada* (mass emergences for oviposition) and yolkless eggs, and so may have a unique reproductive endocrinology.

Gonadotropins

The development of the ovary, its secretion of steroid hormones such as oestrogen and testosterone and ovulation appear to be controlled by follicle stimulating hormone (FSH) or non-specific gonadotropins. Pregnant mare serum gonadotropin (PMSG) (which has primarily FSH activity) was shown by Klicka & Mahmoud (1977) to promote chelonian ovarian growth. Mammalian FSH has been shown to induce ovulation in several species of lizard, and Bentley (1998) assumes that an endogenous gonadotropin has this effect in most reptiles. Licht & Papkoff (1974) specifically demonstrated this in *Chelydra serpentina.* Bentley (1998) reports that pituitary FSH and luteinising hormone (LH) have both been identified in reptiles and negative feedback by oestrogen on the pituitary gland release of gonadotropins is proposed by Bentley (1998).

Calcium metabolism

A variety of hormones, vitamins, nutritional influences and exposure to ultraviolet light (UVB) have a strong influence upon calcium metabolism. These factors act together, and are described both here and elsewhere in this book—with respect to nutrition, husbandry and metabolic bone disease (MBD) (Table 3.8) (Fig. 3.43).

Table 3.8 Factors affecting blood calcium levels.

Factors decreasing blood calcium levels	• Calcitonin is released from ultimobranchial tissue to decrease blood calcium levels • Dietary deficiency of calcium • Starvation • Feeding a diet with a high Ca:P ratio • Dietary deficiency of biologically-available vitamin D • Lack of exposure to appropriate ultraviolet light • Disruption of vitamin D metabolism due to renal, hepatic, intestinal, thyroid or parathyroid disease • High dietary protein may increase calcium excretion
Factors increasing blood calcium levels	• PTH is released to increase blood calcium levels • Increase in blood albumin and associated increase in bound calcium as a result of an increase in receptor sites • Increased intestinal absorption of calcium as a result of the combined actions of vitamin D_3 and PTH • Increased mobilisation of calcium by bone resorption as a result of parathyroid hormone activity

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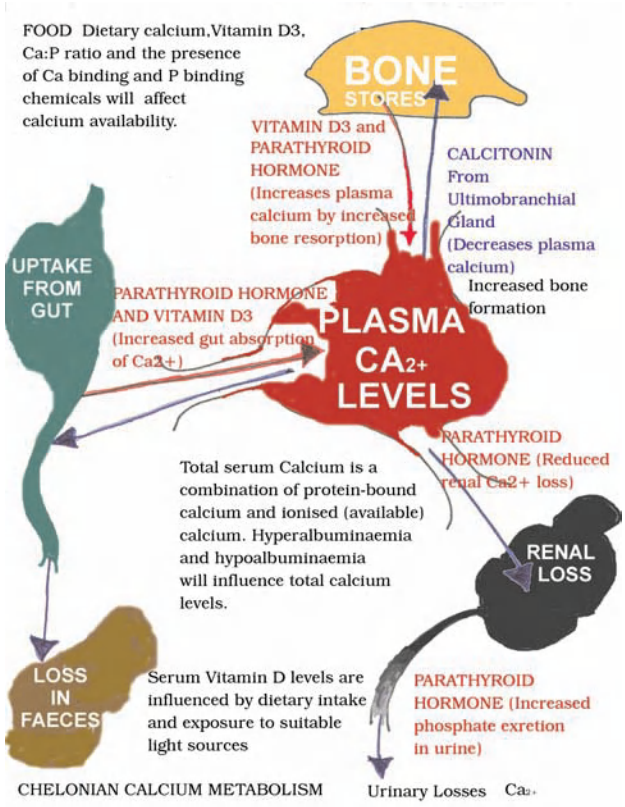

Fig. 3.43 Schematic representation of endocrinological control of calcium metabolism. The importance and influence of ultraviolet light is discussed in the text.

Calcium is involved in four key processes in the body:

(1) it is part of the architecture of bone in combination with phosphorous as a salt;
(2) it has a role in maintaining cell membrane integrity and permeability;
(3) it forms the link between excitation and contraction in muscle and activates secretion within glandular tissue;
(4) it acts as a regulator, activator or inhibitor of key enzymes, such as those involved in clotting.

It is undesirable to have a low blood calcium level. It is associated with muscle weakness, neurological disease, cardiovascular disease, collapse and death in mammals. Similarly, hypercalcaemia is associated with unpleasant consequences such as constipation, anorexia, vomiting, muscle weakness, depression, confusion and coma. Falling calcium levels provoke the release of parathyroid hormone (PTH) and rising levels provoke the release of calcitonin, in order to maintain a physiologically safe serum calcium level.

Blood calcium levels are homeostatically controlled in vertebrates by a combination of the actions of parathyroid hormone, calcitriol (vitamin D) and calcitonin, in conjunction with dietary levels of available calcium and inorganic phosphate. The effects of UVB exposure upon calcium metabolism are explored elsewhere in this book.

Parathyroid hormone (PTH)

The single, rounded thyroid gland lies at the base of the neck ventrally. Chelonians possess four parathyroid glands. The cranial pair is found bilaterally within the thymus glands and the caudal pair lies caudal to the aortic arch in front of the heart. These release parathyroid hormone (PTH). Several workers identify the physiological importance of parathyroid hormone (PTH) in chelonians:

- tri-weekly injections of parathyroid extract (1 USP U/g) in young freshwater turtles (*Trachemys scripta*) produced large osteocytic lacunae and increased osteolytic activity (Belanger *et al.* 1973);
- parathyroidectomy resulted in a significant reduction of serum calcium levels in the turtle *Chinemys reevesii* (Oguro & Tomisawa 1972) and the tortoise *Testudo graeca* (Oguro *et al.* 1974);
- Clarke (1965) did not observe hypocalcaemia in *Chrysemys picta* and *Pseudemys scripta* where two turtles underwent parathyroidectomy. It is possible that removal of parathyroid-secreting tissue was incomplete here, as cells may be distributed elsewhere such as in the lung.

Fowler (1986) describes the actions and control of PTH in vertebrates:

- PTH acts to increase serum calcium levels by increasing bone resorption. This increases calcium release into serum;
- PTH increases renal phosphate excretion;
- PTH increases renal calcium resorption;
- low blood calcium increases PTH release;
- high blood calcium inhibits PTH release.

PTH would also appear to influence other body systems beyond calcium metabolism. In mammals, PTH has been shown to affect lipid metabolism adversely (Akmal *et al.* 1990). If similar effects are seen in reptiles, hyperparathyroidism may potentially trigger off, or predispose towards, hepatic lipidosis in chelonians.

In mammals, excessive PTH is popularly suggested to be nephrotoxic (Slatopolosky *et al.* 1980; Nami & Gennari 1995; Rosol *et al.* 1998; Nagode *et al.* 1996). Endlich *et al.* (1995) and Massfelder *et al.* (1996) have shown that PTH may be a powerful modulator of renal blood flow and glomerular filtration rate.

Slatopolosky *et al.* (1980) propose that PTH may have a role in encephalopathy, increased brain calcium, abnormal EEG, peripheral neuropathy, alterations in lipid, carbohydrate and acid-base metabolism, soft tissue calcification, aortic calcification, impotence, myopathy and anaemia observed in humans with uraemia.

The effects of chronic hyperparathyroidism in chelonians are likely to be undesirable. George (1997) describes loggerhead turtles *Caretta caretta* fed diets consistently low in calcium and high in phosphorous (freeze-dried krill) as hypocalcaemic and hyperphosphataemic. He proposed that the resulting hyperparathyroidism produced demineralisation of bone and pathological fractures.

It would appear likely that chronic hyperparathyroidism may predispose to reduced renal function or even renal failure. This hypothesis is supported by personal observation (SM) of juvenile chelonians with metabolic bone disease (MBD) and the observations of colleagues regarding green iguanas that recover from MBD but subsequently appear to go into renal failure (Frye 1991a; Boyer 1991; Burgmann *et al.* 1993; Redrobe, personal communication 1999).

Hyperparathyroidism may be associated with normocalcaemia and hypophosphataemia. Hyperparathyroidism associated with

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correcting a potential chronic hypocalcaemia may predispose to hepatic lipidosis, soft shell, carapacial deformity and renal disease.

Calcitriol (vitamin D_3)

Vitamin D metabolism in chelonians is poorly understood (Ullrey & Bennet 1999) although these authors review some chelonian data:

- the mean serum 25-hydroxycholecalciferol (25[OH]D) level in adult desert tortoises *Gopherus agassizii* housed outdoors in Nevada was 8.2 ng/ml (n = 14) with a range from less than 5 ng/ml (n = 3) to 16.5 ng/ml (Ullrey & Bernard 1999);
- apparently healthy juvenile desert tortoises and juvenile African spurred tortoises *Geochelone sulcata* housed indoors and fed diets containing about 2000 IU vitamin D_3/kg had serum concentrations of 25[OH]D less than 5 ng/ml. No measurable changes in serum levels were seen after oral dosing with vitamin D_2/D_3 (Bernard 1995).

According to Fowler (1986), in many vertebrates, vitamin D_3 (calcitriol) is produced by synthesis from cholesterol of 7-dihydrocholesterol in the skin. This occurs as a result of exposure to ultraviolet radiation. It is then bound to serum proteins and transported to the liver where it is activated to 25-hydroxycholecalciferol (25HCC). Then 25HCC is transported from liver to the kidney where it is converted to calcitriol (1,25DHCC), the active form of vitamin D. It is not clear if chelonians will have their own methods of transport and activation, as it has been found that thyroxin-binding protein in the plasma of the turtle (*Trachemys scripta*) is also a vitamin D binding protein (Licht 1994). It would seem plausible that interactions between thyroid status and vitamin D status may occur.

The physiological actions and management of vitamin D in vertebrates are reviewed by Fowler (1986):

- vitamin D acts to increase gut absorption of calcium by stimulating active transport across the cellular membrane of the duodenum;
- vitamin D acts together with PTH to promote calcium resorption from bone.

Available evidence would suggest that keepers should expose their reptiles to natural sunlight wherever possible, and to consider the use of UVB-transmitting plastics in enclosure design.

Ullrey & Bernard (1999) review several studies suggesting that some herbivorous reptiles cannot necessarily meet their vitamin D needs by means of oral supplementation. When deficient animals with acceptable dietary levels of vitamin D were exposed to a UVB source (Sylvania Experimental Reptile Lamp) a rise in plasma vitamin D was produced. This rise could not be produced by oral supplementation with vitamin D in similar animals. Dacke (1979) also discusses situations where oral vitamin D is unable to maintain health and UVB supplementation of some reptiles is required. Dacke (1979) also discusses situations where oral vitamin D is unable to maintain health and UVB supplementation of some reptiles is required.

According to Ullrey & Bernard (1999), reptiles like the common iguana, housed differently but otherwise maintained similarly, showed significant differences in their plasma vitamin D levels, with outdoor-housed iguanas at Honolulu Zoo showing dramatically higher levels of vitamin D in outdoor- as compared to indoor-housed specimens. It was proposed that indoor-housed reptiles had avoided the UVB lights provided.

Calcitonin

Calcitonin inhibits bone resorption and decreases calcium release into blood, reversing the action of parathyroid hormone (PTH). It also acts to decrease serum calcium levels. Calcitonin is released by the neuroendocrine C-cells of the ultimobranchial gland (often located within the thyroid gland), in response to high levels of serum calcium (Copp & Klein 1989).

Several workers identify the physiological importance of calcitonin in chelonians:

- Boudbid *et al.* (1987) identified calcitonin in *Trachemys scripta* and found it to have a similar molecular structure to salmon calcitonin;
- Belanger *et al.* (1973) found the administration of 1 ng/g synthetic salmon calcitonin to *Trachemys scripta* blocked the osteolytic effect of parathyroid extract. In these turtles, calcitonin inhibited both osteocytic osteolysis and chondrolysis;
- Klein & Longmore (1986) describe a method of determining calcitonin in reptilian serum;
- Fowler (1986) describes the physiological role of calcitonin in higher vertebrates.

Vitamin-D-binding protein/thyroid-binding protein (DBP/TBP)

It is possible that circulating levels of a protein/hormone (proposed in some texts to be DBP/TBP) influences vitamin D absorption from the gut. Exposure to the appropriate spectrum and intensity of light may affect central production of such a protein/hormone and therefore vitamin D_3 uptake. This hypothesis suggests that appropriate exposure to light may not significantly affect dermal manufacture of vitamin D, but may influence vitamin D uptake from the gut instead.

Thyroid

The chelonian thyroid gland lies ventral to the trachea near the bifurcation of the carotid artery. It is a single organ whose arterial supply is via a branch of the subclavian artery and whose drainage is through the thyroscapular vein (*Chelydra serpentina*).

The thyroid gland occupies a central role in chelonian metabolism. A comparison of thyroid parameters in reptiles and mammals concluded that although the reptilian thyroid is active at high temperatures it is still considerably less active than it is in mammals (Hulbert & Williams 1988).

The main hormones produced by it are tri-iodothyronin (T3) and tetra-iodothyronin or thyroxin (T4). The central role is probably played by T4 as Kohel *et al.* (2001) were unable to detect any measurable T3 levels in all samples taken by them from *Gopherus agassizii* over a two-year period. As in mammals, thyroxin production is controlled by higher centres through thyroid releasing hormone (TRH) and thyroid stimulating hormone (TSH) as well as by melatonin (Sarkar *et al.* 1997).

The effect of thyroid hormones is primarily an increase in metabolic rate in target tissues. Licht (1994) demonstrated an influence of T4 on metabolism of growth in *Trachemys scripta.* T4 tends to enhance the affinity and the capacity for the binding of D_3 at their common transport protein. Thyroid hormones play an important role from early on in the stages of egg incubation. In hatchling snapping turtles (*Chelydra serpentina*) O'Steen & Janzen (1999) found a significant negative correlation between

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incubation temperature and thyroxin plasma levels which induce a paralleled change in metabolic resting rates in the hatchlings. These findings may be important in the evolution of temperature-dependent sex determination.

A number of factors influence thyroid activity. Taking all these variables into account it is difficult to evaluate the chelonian thyroid by laboratory methods, as reference ranges are not always available for different species and sex, as well as for specific temperatures and seasons. TSH or TRH stimulation tests should be evaluated for a specific chelonian species. In order to optimise results, any chelonians tested should be kept under optimal nutritional and environmental conditions for at least 48 hrs.

Temperature

The activity of the thyroid gland is temperature dependent. Using I^{125}, Hulbert & Williams (1988) showed that in *Chelodina longicollis* the uptake of iodine is measurable at temperatures around 21°C and 31°C whereas active secretion is only measurable at the higher temperature. In response to TSH, thyroid glands show little temperature sensitivity *in vitro* in a range between 12°C and 32°C (Licht *et al.* 1989).

The response of peripheral cell receptors to thyroid hormones is also temperature dependent. Comparing metabolic rates, Hulbert & Williams (1988) didn't find any increase in metabolism in animals at 20°C–22°C in contrast to the significant increase in the group kept at 30°C–32°C when thyroxin was injected. Thyroidectomy resulted in a severe decrease in metabolic rate.

Licht *et al.* (1989) showed that the secretion of TSH as a response to TRH is also temperature sensitive. This effect is greatest at preferred body temperature (28°C) and completely suppressed at 5°C–6°C in *Trachemys scripta*. The same authors also concluded that post-receptor events may be more important than binding per se for temperature effects on hormone responses of tissues (Licht *et al.* 1990).

Season

Kohel *et al.* (2001) studied the effect of seasons on plasma thyroxin levels in the desert tortoise (*Gopherus agassizii*). The activity of T4 was lowest during hibernation while a rise in plasma concentration was measurable at the time of emergence. Similar observations were made by Licht *et al.* (1985) for *Chrysemys picta*. T4 levels peaked in females and males in early spring. In addition reproductively-active desert tortoise males showed a second peak during increased mating and combat phase in late summer. This peak was not observed in juvenile males, neither could it be demonstrated in *Chrysemys picta* (Licht *et al.* 1985).

Sex

Licht *et al.* (1990) found that the average plasma T4 levels in *Trachemys scripta* females are higher (137 +/– 17.4 ng/ml) than in males (83.9 +/– 13.8 ng/ml).

Nutrition

Besides seasonal or temperature influences, T4 levels are markedly under the influence of nutrient intake. Withholding of food for a couple of weeks in *Gopherus agassizii* decreases T4 levels significantly (Kohel *et al.* 2001). Once fed again, T4 levels rise within 36 hours.

Species

Norton *et al.* (1989) measured T3 and T4 plasma levels in healthy adult Galapagos tortoises (*Chelonoidis nigra*). T3 levels ranged from 0.33–0.93 ng/ml and T4 levels from 10.8 –1.54 µg/dl. In green turtles (*Chelonia mydas*), mean T4 levels were consistently around 9 ng/ml throughout the year (Licht *et al.* 1985). T4 levels for *Trachemys scripta* are given above. Normal T3 and T4 values are also available for the eastern painted turtle *Chrysemys picta* (Sawin *et al.* 1981).

Hormones

Sarkar *et al.* (1997) demonstrated an inhibitory influence of melatonin on thyroid activity as well as a probable inhibition of thyrotropin release from the pituitary in *Lyssemys punctata punctata*. T4 levels are furthermore controlled by TRH and TSH as is the case in mammals.

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4 NUTRITION

STUART MCARTHUR AND MICHELLE BARROWS

In the wild, starvation and nutritional deficiency may occur as a result of overpopulation and adverse climatic conditions, as well as competition for food sources and habitats with species such as man. However, such situations are unusual, the influence of modern man excepted.

On the other hand, in captivity, deficiencies from inappropriate diets, unbalanced diets and excesses resulting in accelerated growth and obesity are commonplace. George (1997) suggests that wild sea turtles that are able to fulfil their nutritional needs successfully do not exhibit signs of nutritional disease unless a physical or medical problem inhibits their ability to procure food. This is in sharp contrast with the malnutrition and deficiencies of captive-reared sea turtles described later in his paper. Innis (1994) points out that appropriate nutrition of tortoises is essential when raising captive-bred progeny, to allow for proper growth, shell configuration and future reproductive soundness.

Table 4.1 Comparison of annual feeding cycles of free-ranging and captive *Testudo* spp. in the United Kingdom.

Wild chelonians	The availability of food often increases in spring as tortoises awake from winter hibernation, when increasing temperatures and rainfall encourage plant growth. In mid-summer, the intensity of sunshine causes edible plants to dry out. During hot dry periods, many tortoises become inactive and aestivate in burrows. Some show a degree of nocturnal feeding, or feed at dawn and dusk when humidity increases and some plants flourish in response to dew formation. In the autumn, as temperatures fall and rain returns, a further increase in plant growth provides food used to prepare for hibernation. As winter arrives, food availability decreases again. Decreasing temperatures induce hibernation once more.
Captive chelonians	A variety of feeding patterns and environmental cycles are provided to captive chelonians. Some Mediterranean tortoises are maintained all year round in vivaria without hibernation. Some are kept in vivaria and allowed to hibernate for short periods. Some are left out in gardens with no temperature or light supplementation and are encouraged to hibernate for six months or more. A tortoise without light and heat supplementation in the United Kingdom may have only two or three reasonable months a year for feeding.

Most wild chelonians follow an annual cycle of nutrition, which relates to climatic/environmental conditions and physiology. Seasonal periods of activity and feeding in free-ranging tortoises often relate to periods of plant growth, as both cycles are responsive to similar factors, including water availability, temperature intensity, light intensity and photoperiod.

Seasonal alterations in the nutrition of captive chelonians are dependent upon the care of the keeper. This is well demonstrated by a simple comparison of free-ranging and captive herbivorous Mediterranean tortoises (*Testudo* spp.) (Table 4.1).

SELECTION OF AN APPROPRIATE DIET

Chelonians may be carnivorous, omnivorous or herbivorous. Several facultative herbivorous species are suggested to be opportunistically omnivorous (Frye 1991b), however this does not mean they will remain in good health if offered a regular omnivorous diet in captivity (Bone 1992). Generally terrestrial tortoises are either totally herbivorous or are omnivorous (Ernst & Barbour 1989).

Advice concerning supportive feeding of debilitated and hospitalised chelonians is given later in this book. Here we focus on the formulation and organisation of a healthy diet for a normal captive animal (Figs 4.1–4.12). The following tables classify chelonians according to their appropriate diets (Tables 4.2–4.4).

The ideal diet is one that mimics that of the species in the wild as closely as possible. Where necessary, a wide variety of available substitutes should be fed, and over-reliance on a small number of dietary components should be avoided.

Fig. 4.1 Instrument feeding.
A debilitated spur-thighed tortoise (*Testudo graeca*) is placed upon an upturned litter tray to simplify instrument feeding.

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Fig. 4.2 Here a sight-impaired, male spur-thighed tortoise (*Testudo graeca*) is syringe fed during its recovery from 'frost damage' due to an inappropriately managed hibernation. Liquidised food can be offered to the animal, which can learn to accept this form of feeding. Here smell may be an important factor in stimulating appetite.

Early in the management of such animals it may be necessary to open the mouth forcibly and syringe the food in gently until it adapts and assists in the feeding process.

Fig. 4.3 Smell is important in stimulating chelonian appetite, especially where vision is poor, such as in post-hibernation frost damage. Many herbivores are stimulated to eat if food is crushed, allowing fragrances to be released, before placing it before them.

Fig. 4.4 Dandelions provide excellent nutrition for most herbivorous reptiles. They can be picked and stored in a refrigerator before being offered to in-patients.

Fig. 4.5 *Testudo horsfieldi*: These Horsfield tortoises are under treatment for viral stomatitis. During recovery they are offered fresh food in addition to the liquidised food that is administered through their oesophagostomy tubes.

Fig. 4.6 During recovery from disease, the animals in Fig. 4.5 eat easily despite the presence of an oesophagostomy tube. Whilst these tubes are no longer needed to provide nutrition they are being maintained for the administration of medications and fluids.

FEEDING HERBIVOROUS CHELONIANS

Microbial fermentation of ingesta occurs in the large intestine of herbivorous tortoises (Edwards 1991) and marine turtles (Fenchel *et al.* 1979). The advantages of hind-gut fermentation are numerous. Micro-organisms can digest portions of the feed, such as cellulose, that the host cannot. Hind-gut fermentation is dependent upon a favourable intestinal flora and goes some way towards counteracting any amino acid and fatty acid deficiencies initially present in the diet. The gut flora produces additional microbial-derived protein by modifying plant material. The normal digestive flora of most chelonian species commonly encountered in captivity is yet to be determined, but some information has been given earlier in this book.

According to Highfield (1996), the diet of herbivorous tortoises should be:

- high in vegetable fibre which should form the bulk of the diet;
- rich in certain minerals such as calcium;
- rich in vitamins such as vitamin A and vitamin D_3;
- balanced in calcium and phosphorus with more available calcium than phosphorus. It is generally recommended that herbivorous reptile diets should contain a Ca:P ratio of at least 1.5–2:1 (Scott 1996). Wild tortoise diets typically contain a Ca:P ratio of at least 4:1 (Highfield 1994).

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Fig. 4.7 Food preparation for herbivorous chelonians is often easiest where significant numbers of animals are present allowing a variety of foods to be prepared like a salad.

Fig. 4.8 Several varieties of commercial tortoise foods like this one are convenient and very popular with those tortoise keepers attracted to cleverly marketed products for their pets. Excessively high growth rates commonly result from feeding such foods in significant amounts to juvenile animals. Being too high in protein they are a potential source of kidney damage.

Whilst these products are targeted at herbivorous species, this author (SM) feels they do not utilise the normal bacterial fermentation system employed by them to digest and process their food. They appear to contain flavourings (sweeteners) and colourings and therefore in the author's opinion are a poor and potentially inadequate substitute for feeding fresh greens.

Fig. 4.9 Commercial foods are more fibrous, being based around grass/hay, and are far more suited to herbivorous chelonian digestive physiology than the food illustrated in Fig. 4.8. This product is currently under research with a North American animal food manufacturer.

Fig. 4.10 These *Testudo hermanni* hatchlings are aggressively gnawing at chicken bones. These may be a suitable source of calcium, but this author (SM) would prefer a commercial calcium vitamin D balancer such as Nutrobal® (Vetark, UK).
(Courtesy of Frances Harcourt-Brown)

Fig. 4.11 Inappropriate diet: Captive *Testudo hermanni* will readily eat raw meat if it is offered. This species is obviously unable to distinguish between a suitable and an unsuitable diet.
(Courtesy of Frances Harcourt-Brown)

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Fig. 4.12 Inappropriate diet: Captive *Testudo hermanni* will readily eat dog and other animal faeces. In the wild this species is occasionally observed eating dog faeces and invertebrates such as slugs. As a small percentage of dietary intake this may be acceptable, however as management of animal protein intake is so difficult in captive situations, this author (SM) would advise this species is best managed as a strict herbivore in captivity. Should it catch and eat an occasional straying slug then good for both it and the person managing the garden plants! (Courtesy of Frances Harcourt-Brown)

- adequate in water content (water should be available at all times);
- low in certain minerals such as phosphorus;
- low in fats and oils;
- low in proteins;
- low in thiocyanates and oxalates.

In captivity, requirements are most easily met by feeding wild greens such as weeds, flowers and grasses. Complete, pelleted diets are not recommended as a major dietary constituent (see below). Terrestrial herbivorous tortoises are normally fed daily. All chelonians must be given regular access to fresh water for drinking and bathing. Edwards (1991) determined that the dry matter digestibility of dried grasses, sedges and herbs was 30% and the gross energy digestibility 34.5%. Edwards advised that legume or grass hay was well suited to Aldabran tortoises and other large tortoises such as *Geochelone pardalis* and *G. sulcata,* and that this can be balanced by the addition of an alfalfa-based herbivore pellet. Most keepers must utilise grocery greens to some extent but it is important to appreciate that these items are generally higher in protein and lower in fibre compared to natural forage, and, in many cases, have an inverse Ca:P ratio.

Table 4.2 Terrestrial tortoises classified according to diet.

Herbivorous	• Hermann's tortoise (*Testudo hermanni*) • Afghan/Steppe tortoise (*Testudo horsfieldi*) • marginated tortoise (*Testudo marginata*) • spur-thighed tortoises (*Testudo graeca ibera, Testudo graeca graeca, Furculachelys whitei etc.*) • Egyptian tortoise (*Testudo kleinmanni*) • African spurred tortoise (*Geochelone sulcata*) • leopard tortoise (*Geochelone pardalis*) • radiated tortoise (*Asterochelys* or *Geochelone radiata*) • Aldabran tortoise (*Geochelone gigantea*) • North American gopher tortoises (*Gopherus polyphemus, Gopherus flavomarginatus, Gopherus agassizii, Gopherus berlandieri*) • Argentine tortoise (*Geochelone chilensis*) • yellow-foot tortoise (*Geochelone denticulata*) • Indian star tortoise (*Geochelone elegans*) • pancake tortoise (*Malacochersus tornieri*)
Mainly herbivorous (a very small intake of animal matter and insects)	In the wild, certain essentially herbivorous species appear to take small numbers of invertebrates and carrion. • (Frye 1991b) categorises *Testudo hermanni* as 'eating animal matter' and many healthy adult *T. hermanni* regularly eat slugs. However, this author (SM) would advise that this species be managed as though a herbivore when in captivity. • The main diet of the red-foot tortoise (*Geochelone carbonaria*) is fallen fruits, leaves and flowers, with occasional carrion. • Padloper tortoises (*Homopus* spp.) occasionally eat hyena faeces, snails and beetles. • The Burmese brown tortoise (*Manouria emys*) is primarily herbivorous but consumes a small amount of animal matter. • The bowsprit tortoise (*Chersina angulata*), the spider tortoise (*Pyxis arachnoides*), the flat-tailed tortoise (*Pyxis planicaud*) and the impressed tortoise (*Manouria impressa*) may ingest invertebrates, either directly or indirectly, associated with fruits or fungi.
Omnivorous	• African hingeback tortoises (*Kinixys* spp.) • North American box turtles (*Terrapene* spp.) • some Asiatic box turtles (e.g. *Cuora galbinifrons*) • jagged-shell turtle (*Pyxidea mouhotii*) • black-breasted leaf turtle (*Geoemyda spengleri*) • elongated tortoises (*Indotestudo* spp.) • South American wood turtles (*Rhinoclemmys* spp.) • With omnivorous species the preference for animal or plant matter usually varies with life stage. Young animals tend to be more carnivorous and insectivorous than adults. Often such species are forest ground dwellers living in high humidity and semi-darkness, where slugs and insects are plentiful.

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Table 4.3 Semi-aquatic chelonians classified according to diet.

Herbivorous	Few semi-aquatic chelonians are herbivorous, the Indian roofed Turtle (*Kachuga tecta*) being a potential exception. Adults of several North American Emydid turtle species, e.g. the red-bellied turtle (*Trachemys rubiventris*), are primarily herbivorous.
Omnivorous	Most semi-aquatic species are omnivorous with the specific diet varying according to location and habitat: • painted turtle (*Chrysemys picta*) • spotted turtle (*Clemmys guttata*) • wood turtle (*Clemmys insculpta*) • bog turtle (*Clemmys muhlenbergi*) • Malayan box turtle (*Cuora amboinensis*) • Asian leaf turtle (*Cyclemys dentata*) • Blandings turtle (*Emydoidea blandingi*) • red-bellied short-necked turtle (*Emydura subglobosa*) • map turtles (*Graptemys* spp.) • giant Asian pond turtle (*Heosemys grandis*) • cog-wheel turtle (*Heosemys spinosa*) • mud and musk turtles (*Kinosteron* spp.) • diamond-backed turtle (*Malaclemys terrapin*) • Indian black turtle (*Melanochelys trijuga*) • Mediterranean and Asiatic pond turtles (*Mauremys* spp.) • South American side-necked turtles (*Phrynops* spp.) • black marsh turtle (*Siebenrockiella crassicolis*) • red-eared slider(*Trachemys scripta elegans*) • pig-nosed turtle (*Carettochelys insculpta*) and the giant Asian river turtle (*Batagur baska*) are essentially herbivorous, although some fish, crustaceans and molluscs are consumed • South American side-necked turtles (*Platemys* spp.), reversing the usual trend, as juveniles are more herbivorous than adults • Sulawesi forest turtle (*Leucocephalon yuwonoi*)
Carnivorous or predominantly carnivorous	• European pond turtle (*Emys orbicularis*) • snapping turtle (*Chelydra serpentina*) • Australian side-necked turtles (*Chelodina longicollis*) • Matamata (*Chelus fimbriatus*) • Argentine side-necked turtle (*Hydromedusa tectifera*) • African helmeted turtle (*Pelomedusa subrufa*) • African side-necked turtles (*Pelusios* spp.) (predominantly carnivorous) • big head turtle (*Platysternon megacephalum*) (predominantly carnivorous) • soft-shelled turtles (*Trionyx* spp.)

Table 4.4 Marine turtles classified according to diet.

Marine turtles	Juvenile sea turtles generally feed on fish, molluscs, coelenterates (jellyfish) and marine vegetation, but as they approach adulthood most become essentially herbivorous (Frye 1991b). Bjorndal (1997) breaks down the dietary preferences and foraging ecology of various sea turtles (*Caretta caretta, Chelonia mydas, Eretmochelys imbricata, Lepidochelys kempi, Lepidochelys olivacea, Natator depressus and Dermochelys coriacea*) using available literature describing faecal analysis, stomach lavage and stomach-content analysis of wild specimens. Most species showed evidence of an omnivorous diet at some stage in their life cycle, but the flatback *Natator depressus* showed little evidence of herbivorous foraging and the diet of the leatherback *Dermochelys coriacea* is markedly pelagic.

General advice for feeding herbivorous tortoises

- Encourage natural foraging and the use of wild-picked weeds and grasses. Tortoises forage for themselves if provided with a suitably-planted large enclosure, but will usually require additional food. Beware of poisonous plants (such as daffodils, potatoes, buttercup and yew), a comprehensive list of which is given in Appendix B.
- In order to reduce selective feeding, offer food blended together in mixtures. Various ingredients can be mixed with a vitamin/mineral supplement, balancing components such as calcium, iodine, vitamin D_3 and vitamin A. This is offered fresh, on a daily basis, as a sort of raw coleslaw. Daily supplementation with a suitable vitamin and mineral preparation is advised in most nutritional and husbandry texts.
- Washing all food is advisable, as many ingredients may have been sprayed with pesticides to which chelonians may be sensitive. Similarly, it would be wise to use organic produce if buying grocery greens.
- Theoretically, oxalates found in spinach, cabbage and beet greens bind calcium, reducing its availability to the tortoise, and goitrogens found in cabbage and kale appear to have caused hypothyroidism in giant tortoises (Frye 1991b). However, these items are unlikely to result in problems if used as components in a varied diet with supplementation as discussed below. It is likely that these points have been overemphasised in earlier texts and many herbivorous chelonians will tolerate such foods well, if not given to excess.
- Rhubarb should never be fed to chelonians (Frye 1991b).
- Little is known about the true suitability of existing commercial dried/pelleted tortoise diets at the time of writing. This author (SM) suggests that they should be avoided, especially in juvenile animals where mistakenly accelerated growth and selective feeding may be encouraged. Their use in adults is also of dubious benefit. Most of these pellets contain very high protein levels, up to 45%, which would have a detrimental effect as described later. Perhaps in time a variety of suitable pelleted foods may become available. Recent work feeding

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large tortoises such as *Geochelone sulcata* and *Geochelone pardalis* on high-fibre pelleted timothy hay has been promising. Commercial foods should be offered or rejected in line with the data made available to validate their suitability.

- This author (SM) suggests that we should not offer animal protein to herbivorous chelonians for a variety of reasons. Animal protein bypasses the normal process of hind-gut fermentation, can derange gut flora and predisposes to hyperuricaemia and therefore gout. It may also result in accelerated and abnormal growth patterns in juveniles. Parallels to the scrapie transfer encountered when herbivorous farmed mammals were fed animal protein may also be possible.
- Cat food, dog food, bread and milk, mammalian veterinary nasogastric tube solutions, mammalian recovery diets (e.g. Hills a/d, Colgate Palmolive; Reanimyl, Virbac) and human milk-based nutritional compounds (such as Complan), cheese, baked beans, peas, sweet corn and bacon are all unsuitable for *herbivorous* tortoises. Bread is occasionally well tolerated but is a questionable dietary component.
- When nursing debilitated and anorectic herbivorous chelonians this author (SM) has met with great success using Critical Care Formula (Vetark, UK) and the normal diet in liquidised form, as both pass readily through common gavage and oesophagostomy-placed feeding tubes.

Suitable dietary components

The following dietary components are suggested at our surgery as being suitable and readily available to those maintaining captive herbivorous chelonians (Table 4.5).

Food analysis

Below are analyses of some of the more common foods fed to captive herbivorous reptiles (Tables 4.6 and 4.7).

Table 4.5 Dietary components suitable for captive herbivorous chelonians.

Category	Amount to be fed	Examples	
Green-leaf base	Green-leaf base should comprise 75–95% of the normal diet of Mediterranean tortoises such as *Testudo hermanni* and *Testudo graeca*.	• dandelion: leaves and flowers • alfalfa : fresh, sun-cured hay, dried leaves, pellets • mixed grasses: fresh, sun-cured hay, dried leaves, pellets • cabbage (mixed varieties) • rocket • clover shoots	• kale • rape • parsley • watercress • spring greens • carrot tops • beet tops • sowthistle • turnip tops • chickweed
Vegetables	5%–15% of the diet of Mediterranean tortoises should be grated or chopped vegetable matter.	• beans (leaves and pods) • broccoli • Brussels sprouts • cauliflower • beetroot	• carrot • parsnips • turnip • marrow • pumpkin
Fruits and succulents	Fruits should be fed cautiously. High sugar levels can encourage bacterial, mycotic and protozoan overgrowth. This is particularly likely following antibiotic treatments. Fruit should form no more than 10% of the normal diet of Mediterranean tortoises.	• melon • tomato • mango • apple • pear • peppers	• cucumber • grapes • mulberry • peach • apricot • nectarine
Garden forage	It is essential to remove any potentially toxic plants from the garden and to avoid the use of any chemicals such as pesticides and slug pellets. It is also important to retrieve tortoises before mowing the lawn!	• lawn grass, clovers and dandelions • hibiscus • mint • nasturtium	• lilac • rose • bramble • flowers and their leaves
Wild plants (Highfield undated)	Care should be taken to ensure that poisonous plants listed later are not mistakenly offered.	• dandelion • clovers • hawkbits • sowthistles • hawkweeds • mallows • bindweeds • sedum • ivy-leaved toadflax • honeysuckle	• cats' ears • vetches • trefoils • bramble • chickweed • dock • plantain • nettles • hedge mustard

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Table 4.6 Energy and nutrient content of some suitable foods for herbivorous reptiles (Donoghue & Langenberg 1996; Donoghue 1996).

Food item	Weight g	Water %	Energy As fed cal/g	Energy Dry Matter	Protein % DM	Fat % DM	Carbohydrate % DM	Fibre % DM	Ca % DM	P % DM
Greens										
romaine lettuce	100	94	0.18	3.0	36	7	50	11	1.1	0.4
iceberg lettuce	100	96	0.13	3.2	25	0	59	11	0.4	0.5
spinach	100	91	0.26	2.9	36	3	48	7	1.0	0.6
dandelion greens	100	86	0.44	3.1	18	5	61	11	1.2	0.4
beet greens	100	91	0.24	2.7	24	3	51	14	1.3	0.4
alfalfa sprouts	100	88	0.39	3.2	37	4	39	12	0.3	0.8
mung bean sprouts	100	89	0.35	3.2	31	2	54	6	0.1	0.5
Vegetables										
mushrooms	100	90	0.27	2.7	30	6	49	9	0.1	1.3
frozen mixed vegetables	100	83	0.47	2.8	16	2	68	7	0.1	0.3
Fruit										
apple	128	84	0.51	3.2	1	2	86	4	trace	trace
banana	114	74	0.82	3.2	4	2	86	2	trace	trace
strawberries	149	92	0.28	3.5	6	4	77	6	0.2	0.2

Table 4.7 Calcium and phosphorus levels of some fruits and vegetables (Jackson & Cooper 1981b).

	Calcium mg/100g	Phosphorus mg/100g	Ca:P ratio
lettuce	25.9	30.2	0.86:1
tomato	13.3	21.3	0.62:1
cucumber	22.8	24.1	0.95:1
broccoli tops	160.0	54.0	2.96:1
cauliflower (boiled)	23.0	33.0	0.69:1
carrots (boiled)	36.9	16.7	2.21:1
watercress	222.0	52.0	4.27:1
melon	13.8	8.7	1.59:1
grapes (white)	19.1	21.9	0.87:1
grapes (black)	4.2	16.1	0.26:1
cherries	15.9	16.8	0.95:1
dried apricots	92.4	118.0	0.78:1
pears	6.9	9.5	0.72:1
apple	3.6	8.5	0.42:1
banana	6.8	28.1	0.24:1

FEEDING OMNIVOROUS TORTOISES AND SEMI-AQUATIC CHELONIANS

Omnivorous tortoises

Donoghue (1996) suggests omnivores do well in captivity when fed plant and animal matter in proportions ranging from 75:25 to 90:10. Some preferences exist and these differ with species and habitat. The reader is also encouraged to seek published articles for the species of interest and the advice of experienced keepers.

Most omnivorous chelonian juveniles are particularly carnivorous but there is a tendency for a higher proportion of vegetable matter to be consumed as they mature. McCauley & Bjorndal (1999) looked at the relationship between gut capacity and metabolic rate and concluded that processing limitations imposed by small body size do not constrain juvenile *Trachemys scripta elegans* from adoption of an herbivorous diet.

In the wild, omnivorous species will thrive on a variety of live and often insect foods, including earthworms, slugs, snails, millipedes, woodlice, pupae and maggots. The health of captive-bred insects and worms should be carefully maintained. Frye (1991b) gives simple advice regarding the breeding of such food. In order to reduce the likelihood of nutritional disease in animals eating them, insect pupae and larvae should be offered a diet of vitamins and minerals only, in the 24 hrs before their consumption, so that they become 'gut loaded'. Subsequently they should be dusted with appropriate vitamins and minerals immediately prior to feeding. Since most larvae have an excess of phosphorus, supplements fed to them should be high in calcium to preserve the Ca:P ratio.

Occasionally, a small amount of meat, fish or low-fat dog food may be offered to most omnivorous species. The fat content of some cat and dog foods is particularly high and these should be avoided. A balanced and varied diet is better than an addiction to a limited variety of foods, and dog food should only be fed as a moderate proportion of a more balanced and varied diet. The frequency with which animal protein should be offered to omnivorous species depends upon life stage and the degree to which the species is carnivorous. Although many commonly encountered species tolerate low-fat cat or dog food once a week, the natural diet should always be considered superior.

Frye (1991b) describes the pathogenic effects of feeding excessive amounts of cat food, dog food and monkey chow. He explains that high levels of vitamin D_3 appear to encourage soft tissue mineralisation, described later as *metastatic calcification*.

Many omnivores will refuse vegetable and fruit matter if it is offered in a fresh state, with preference shown for fallen and rotten food. Experimentation may be necessary when a new specimen is acquired, especially if it is wild caught. Even within species a great deal of variation in habitat type and diet occur in the wild.

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Semi-aquatic chelonians

Nearly all semi-aquatic chelonians are omnivorous and thrive on both animal and plant material in suitable combinations. Commercial turtle flakes with high proportions of dried shrimp may be lacking in minerals and vitamins, whilst feeding oily fish in large quantities may result in steatitis/fatty liver and B vitamin deficiency due to an excess of thiaminases. A suitable diet will consist of a range of products that complement each other. A small amount of low-fat dog food can be offered occasionally: about once a week is suggested in many texts. Again the natural diet should always be considered to be superior whenever it is available.

Some of the Asian box turtles, such as the Chinese three-striped box turtle (*Cuora trifasciata*) and the Malayan box turtle (*Cuora amboinensis*), prefer to be fed in water.

Diets containing significant levels of animal protein tend to have a high phosphorus level in relation to calcium and this may well result in abnormal shell and bone growth. Supplementation with some accessible form of calcium (such as Nutrobal®, Vetark) will counteract this, but this is hard to achieve when the animals only feed in water.

Diets suitable for omnivorous chelonians

Box turtles

Boyer (1992c) comments that many box turtles are far more carnivorous than their keepers realise. He suggests that one third to one half of the diet should be plant based. The other half to two thirds of the diet should be animal-matter based. Of the plant-based portion he advises 70%–80% should be vegetable based and 20%–30% fruit based. He advises that cat food should be avoided and dog food and monkey chow should not make up more than 5% of the total diet. He further advises that liver, along with yellow or dark-orange coloured vegetables (such as squash, carrots and sweet potatoes), is an excellent source of vitamin A. Rhubarb should be avoided (Table 4.8).

Box turtle hatchlings feed almost exclusively on the small, live prey listed above, while mature animals also require fruit and vegetables.

Table 4.8 Components of a suitable diet for a captive box turtle (Tortoise Trust).

Greens	Fruits	Animal protein
collard greens	apples	dog food (semi-moist, canned or soaked dry)
mustard greens	grapes	whole, skinned, chopped mice
radish	peaches	pinkies
turnip/beet tops	melon	trout chow
kale	plums	sardines
bok choy	strawberries	earthworms
escarole	banana	crickets
spinach	pears	waxworms
chard		slugs
Savoy/romaine lettuce		pupae
dandelions		maggots
broccoli		woodlice
mushroom		other insects

Some other terrestrial and semi-aquatic omnivores

African hingebacks (*Kinyxis* spp.) feed on a variety of animal and vegetable foods (Innis 2000):

- live worms, slugs, snails, millipedes, woodlice and other invertebrates;
- pinkies;
- skinned, chopped, adult mice;
- general vegetation and grasses;
- fallen fruit;
- mushrooms, lettuce;
- bananas, peaches, tomatoes.

Semi-aquatic omnivores, such as the red-eared turtle (*Trachemys scripta elegans*), also thrive on a mixed diet:

- turtle sticks and flakes;
- plant leaf material;
- fruit;
- canned dog food;
- rehydrated cat or dog pellets;
- pond fish pellets;
- raw whole fish;
- fresh meat (liver);
- insects.

Many aquatic and semi-aquatic chelonians must be fed in water. They vary from the highly carnivorous matamata (*Chelus fimbriatus*), soft-shelled turtles (*Trionyx* spp.) and snapping turtle (*Chelydra serpentina*), to the omnivorous red-eared slider (*Trachemys scripta elegans*), which, as an adult, is primarily herbivorous. Suitable dietary constituents may include greens and fruit, as for herbivorous tortoises, pond weed, pond-fish pellets, insects, bloodworms, tubifex worms, raw whole fish, prawns, pinkie and chopped adult mice and small amounts of fresh meat.

Low-fat dog food can be fed in small amounts. Rehydrated pellets are preferable to tinned food, as they will cause less water pollution. Cat food is less suitable due to the higher fat content. Of the pelleted or flaked turtle diets available, ReptoMin® sticks (Tetra) are recommended.

Again, the key to nutritional management is to avoid over reliance on one or two items. Turtles can easily become addicted to meat, fish or prawn-only diets with resultant nutritional imbalance and disease. Whole fish are preferable to gutted fish and adult mice are nutritionally superior to pinkies.

Supplementation with a high calcium and vitamin powder, as for terrestrial species, is advised. It can be administered by dipping wet items in the powder and feeding directly from a pair of forceps, by rehydrating dog or trout pellets in water containing the supplement or by making up feeding cubes consisting of liquidised food items and the required supplement using gelatine powder. The mixture is set and then frozen to use as required. It is advisable to use a separate feeding tank or bowl to reduce contamination of the main tank.

Adult aquatic chelonians should be fed no more than three or four times a week, as obesity is common in captive animals.

Aquatic sea turtles

George (1997) describes the successful rearing of captive-farmed turtles using commercial pelleted food, modified pelleted trout

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ration and balanced gelatine diets. Commercial diets generally ranged from 25%–35% protein, but a 45% protein diet has been used with success in Kemps Ridley turtles. The requirements of hatchling green turtles (*Chelonia mydas*) for seven amino acids have been determined and a requirement for vitamin A suggested (Bjorndal 1997). Diets for captive sea turtles restricted to freeze-dried krill, or squid and fish, have been implicated as the cause of nutritional disorders (George 1997).

VITAMIN, MINERAL AND TRACE-ELEMENT SUPPLEMENTATION

Jackson (1982) and Divers (1996b) point out that captive chelonians fed appropriately should not require supplementation with minerals and vitamins. As most are not kept under optimal conditions, they recommend that all captive chelonians should have appropriate supplementation to counteract limitations in both diet and environment. Donoghue (1995a) points out that veterinarians may be professionally liable for any harm that results from the use of nutritional supplements which they have recommended, and care should be taken where there is a narrow margin of safety.

Iatrogenic hypervitaminosis A is a potentially fatal condition, readily induced with injectable preparations, particularly aqueous solutions. Therefore, appropriate care should be taken in calculating dose rates. In many situations oral supplementation will be entirely adequate, particularly in terrestrial chelonians. Hyper- and hypovitaminosis A are discussed in detail later, with reference to nutritional disease. Below are some vitamin supplements with their vitamin A content listed (Table 4.9).

Jackson (1982) suggests that the following various vitamins, minerals and trace elements should be fed to terrestrial chelonians, preferably as a balanced diet from a selection of appropriate food sources:

- vitamin A (bone formation and prevention of hypovitaminosis A);
- vitamin D_3 for proper bone development;
- vitamins B_1, B_2, B_6 and B_{12} for growth, cellular metabolism, central nervous system (CNS) function and erythropoiesis;
- calcium pantothenate and folic acid for growth and appetite;
- vitamin E for reproduction and muscle development;
- choline chloride to help fat metabolism;
- menadione for the production of prothrombin;
- calcium and phosphorus in appropriate balance which is generally believed to be greater than 1:1 and possibly as high as 4:1 in wild grazing tortoises (Highfield 1996);
- iron, copper, cobalt, manganese, magnesium, sodium and iodine.

Table 4.9 Common oral vitamin A supplements.

Trade name	Vitamin A content
Abidec drops® (Warner Lambert)	4000 IU /0.6ml
Vionate®, (Vetark, UK)	220,000 IU/Kg
dandelion leaves	14 000 IU/100g (Highfield 1996)
VitanA Palmitate® (Cambridge Labs, UK)	50 000 IU/ml
Aquasol A® (injectable) (USA)	50 000 IU/ml

Juveniles

Inappropriate food and inadequate environmental provision of suitable UVB are common in juvenile tortoises presented at this author's clinic (SM). Yolk sac reserves of essential nutrients are quickly exhausted and juvenile chelonians fed inappropriately are highly susceptible to nutritional disease. It is wise to encourage a constant, low-level provision of an appropriate vitamin supplement. This should contain calcium and vitamin D_3 to achieve satisfactory skeletal growth and good levels of dietary vitamin A.

Frye (1991b) suggests that in semi-aquatic chelonians a period of up to six months may elapse before yolk-sac vitamin A is exhausted. It is at this stage that clinical signs of deficiency may suddenly be seen. Therefore a hatchling turtle may have been in the care of the keeper for nearly six months before the inadequacies of any diet fed may become apparent.

Adults

With suitable feeding, the need to supplement the diet of adult chelonians is limited, outside of the breeding season in mature females, although it is unlikely that moderate oral supplementation of adult chelonians will cause harm in any case (metastatic mineralisation is discussed later). If UVB lighting is not provided and a restricted diet is offered, supplementation with an all-round supplement such as Arkvits® (Vetark, UK) is advised. Chronic hyperparathyroidism is likely where chelonians are offered poor lighting and inadequate calcium and vitamin D_3.

Reproductively-active females

Calcium demands increase with the demand for calcification of ovulated eggs. Blood calcium and albumin measurements show apparent elevations during periods of folliculogenesis due to Ca^{2+}/albumin binding. At this time females will actively seek out and eat white material such as china, stones and bone. Such behaviour predisposes to ingestion of foreign bodies. It is wise to add a balanced calcium and vitamin D_3 supplement to the diet at this time (Table 4.10).

PROTEIN

Sources of protein

Donoghue (1996) points out that generalisation about protein sources in chelonians is risky, given the shortage of published data. She suggests that plant proteins often lack the essential amino acids lysine, methionine, cystine, tryptophan and threonine, and that meats contain high levels of fat, phosphorus and purines. Many herbivorous chelonians rely on hind-gut degradation of cellulose-based material and the production of fresh sources of microbial protein (Skoczylas 1978). This means that excessive analysis of dietary protein may be misleading, as protein content will be substantially altered during digestion.

Quantity of protein

Too much protein is bad, not enough protein is bad, and the wrong sorts of protein are bad. Where possible, refer to what

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Table 4.10 Components of some common reptile dietary supplements available in the United Kingdom (Scott 1992; Divers 1996c).

	Vitamin A (IU/g)	Vitamin D_3 (IU/g)	Calcium (mg/g)	Phosphorus (mg/g)	Ca:P ratio
Nutrobal® (Vetark, UK)	500	150	200	4.5	46:1
ACE High® (Vetark, UK)	2530	20	9.9	4.9	2:1
Arkvits® (Vetark, UK)	1177	118	142	4.65	30:1
Vionate® (Ciba Geigy)	220	22	94.5	63.6	1.46:1
SA 37® (Intervet)	769	76.9	10	10	1:1

would be the typical protein intake of each species in the wild and mimic it. During the growth periods of hatchlings and juveniles, food restriction avoids excessively high growth rates. We regularly suggest alternate-day feeding and restricted feeding periods where growth is excessive.

Frye (1991b) advises against offering herbivorous chelonians animal protein, as it will predispose towards hyperuricaemia and therefore gout. Diseases associated with high protein diets such as accelerated growth, early maturity, hyperuricaemia and gout are all described in more detail later. Innis (1994) points out that, anecdotally, excessive protein during the first years of life is considered a cause of 'pyramiding' of the scutes. He also suggests that excessive protein may reduce fat usage as protein becomes involved in gluconeogenesis and therefore increasing relative available fat may predispose towards hepatic lipidosis. An abnormal diet might disrupt normal gut flora and destabilise the normal digestive process.

NUTRITIONAL DISEASE IN CAPTIVE CHELONIANS

Wallach (1971) gives the average life expectancy of a captive reptile in the United States as less than two years. In reptiles managed completely inappropriately, he suggests that body energy and essential nutrient stores may last for up to this period and points out that it is only after this time, when such reserves have been depleted, that reptiles succumb to nutritional diseases or secondary infections.

In the United Kingdom, high mortality rates for juvenile *Testudo* spp., hatched and raised in captivity were reported by Lambert (1986 & 1988). In his study, the median survival rate of *Testudo graeca* was 1.5 years, *T. hermanni* was 1.75 years, and *T. marginata* was 2.3 years. Inappropriate husbandry and nutritional disease were considered major contributing factors. According to Highfield (1988), dietary disorders of juvenile chelonians are a rapid and major cause of mortality in United Kingdom captive-bred hatchlings. In adults he suggests that nutritional disease is a less acute problem, with inappropriate nutrition and husbandry tolerated for five or ten years before disease is apparent to the keeper.

At this author's clinic (SM), nutritional disease of captive chelonians appears to be very common. Cases are usually chronic unless the animal is a juvenile, and they are often presented by clients who have fed diets containing limited ingredients without supplementation of vitamins and minerals. Many diets are often based around the preference of the animal or human convenience foods. Often, clients presenting chronically-ill chelonians have received no husbandry or nutritional advice in the 20 or so years in which they have cared for their pet. Such keepers regularly state that a children's television programme, *Blue Peter*, was the entire source of their chelonian care information. In most cases the client is unaware of any nutritional problem. Conditions such as cloacal organ prolapse or soft shell are rarely considered to be of potential nutritional origin by clients until veterinary consultation.

A number of studies have been undertaken to discover the prevalence of nutritional disease in captive chelonians:

- Jackson (1980a) found that 31% (25 freshwater chelonians and 6 terrestrial tortoises) of 100 chelonians presented at his surgery were suffering from nutritional osteodystrophy. Hypovitaminosis A was proposed in 5% of cases;
- Keymer (1978b) found that nutritional disease affected 19.7% of the freshwater chelonians in his necropsy survey. Osteopathies affected 19% and 9.8% were considered exclusively nutritional. Hypovitaminosis A was suspected in 3.3% of cases;
- Jacobson (1994) surveys the literature describing conditions such as metabolic bone disease, hypovitaminosis A, toxicities and hypothyroidism and comments on their relevant prevalence;
- Dollinger *et al.* (1997) reviewed the husbandry and pathology of terrestrial tortoises in Swiss zoos and found hepatic lipidosis, goitre, visceral gout, oesophageal calcification and *osteodystrophia fibrosa*, with metabolic diseases being the most frequently encountered disease group.

Common nutritional diseases and their signs

Maintaining a reptile in a well-illuminated and heated vivarium on an excellent level of nutrition is the aim of all caring chelonian keepers. However, potential complications of such high levels of husbandry, such as accelerated growth, early maturity, carapacial deformities, metabolic bone disease and gout should always be considered when keeping reptiles.

Nutritional diseases which are well defined in the literature such hypovitaminosis A, hypo-iodinism, metabolic bone disease/nutritional secondary hyperparathyroidism, gout and hepatic lipidosis are described under specific headings in later sections (see 'Problem-solving approach to common diseases of terrestrial and semi-aquatic chelonians' and 'Problem solving approach to conditions of marine turtles', pp 301–377). The table below summarises some of the more common nutritional diseases, their signs and how to correct them (Table 4.11).

Overfeeding

Many keepers present overweight tortoises to veterinarians after only a day or two of anorexia. Similarly the feeding periodicity of

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Table 4.11 Some common nutritional diseases, their causes and cures (adapted from Donoghue 1995a).

Disease/clinical sign	Nutritional imbalance	Nutritional cause	Corrective measures
Cachexia	Deficiency of energy	Starvation; low food intake (possibly due to selective feeding)	Increase ambient temperature; provide more food; alter food composition; possibly increase amount of grass and greens fed
Obesity	Excess of energy	Excess of food; lack of activity	Increase activity required to find food; increase proportion of low energy-dense foods; decrease dry matter intake by 10%, 20%, 30% and 40% over successive weeks
Metabolic bone disease	Calcium deficiency; vitamin D_3 deficiency	Low calcium food; no sunlight or UVB irradiation	Supplement with calcium salts, legumes, etc.; house outside; assess UVB provision and improve it
Hypovitaminosis A	Vitamin A deficiency	Restricted diet, e.g. lettuce only	Improve dietary provision and supplement with vitamin A
Hypervitaminosis A	Vitamin A excess	Overdose of vitamin A (usually parenteral)	Stop supplementation
Steatitis	Vitamin E deficiency	Excess polyunsaturated fatty acids	Add vitamin E; alter diet
Neurological signs	Potential thiamine deficiency	Usually a fish-only diet in an omnivorous or carnivorous species	Improve diet; vary fish species fed; add thiamine to diet
Gout	Purine excess; water deficiency	Excessive offal; dehydration through inadequate water provision	Decrease levels of purine in the diet; improve fluid availability and administration
Goitre	Iodine deficiency or excess	Iodine deficient soil; excessive supplementation	Add or reduce iodine supplements; remove goitrogens from the diet.

captive reptiles is poorly understood. In the wild, perfect feeding conditions do not exist. Adverse weather will result in periods where feeding is restricted. At these times a tortoise may need to graze a large area in order to obtain suitable food. Sometimes they must simply wait until conditions improve before feeding is resumed. These feeding cycles are not easily reproduced in captivity. Most caring keepers place copious amounts of high-quality food directly before the mouths of their reptile, which may not need to move at all in order to feed. Without some attention, this situation predisposes to obesity.

This author (SM) considers it wise to reduce both the amount and frequency of feeding in captive chelonians where exercise is restricted and obesity can easily be predicted. This is especially true of excessively well-maintained juvenile terrestrial chelonians.

Early maturity and accelerated growth in juvenile terrestrial tortoises

Environmental conditions provided to many growing tortoises maintained in captivity by caring keepers favour accelerated growth rates. In captivity, fast growth rates can be obtained in juveniles and hatchlings by feeding diets with a high protein content (Jackson *et al.* 1976; Lambert 1986; Reid 1986; Lambert 1988). However, it is unwise to offer high protein diets to herbivorous juvenile chelonians, as high growth rates are not always beneficial. High growth rates achieved by feeding proprietary tortoise diets, peas, beans, meat and dog food can result in growth defects and disease (Bone 1992).

We do not yet have access to validated growth-rate curves for most species in captivity under optimum husbandry conditions. Optimum husbandry conditions for many species are unknown and are frequently areas of heated keeper dispute. In the next decade it is likely that peer reviewed/validated reference growth rate curves and the husbandry data necessary to achieve optimum growth of neonates will become available. This author (SM) would recommend that their use is encouraged and used by veterinarians in order to give clearer guidelines to keepers regarding the care of juvenile chelonians.

Excessive growth rates in juvenile chelonians are associated with high mortality, renal disease and obvious irreversible deformities of the skeletal system and this can manifest itself rapidly in juveniles. Abnormal growth of the carapace is the most obvious sign: the scutes tend to change in angle resulting in so-called *pyramiding*. The shell often appears undersized compared to the limbs and head. Findings reported when accelerated growth is also concurrent with inappropriate lighting, restricted diet and dietary deficiency of physiologically useful calcium and vitamin D_3 include a softening of the plastron, leg weakness, carapacial deformity, renal failure and even death. Young tortoises fed on unsuitable high-protein diets, such as beans or meat, often demonstrate remarkable growth rates. Renal failure may often hinder treatment attempts.

Pyramiding and abnormal calcium metabolism are not inevitably linked. Fast-growing animals with excellent calcium, vitamin D_3 and UVB provision may also grow abnormally. This is especially true where hibernation is avoided and high quality foods and long winter photoperiods are provided. Often vivaria are illuminated with the best quality UVB lighting and maintained at favourable temperatures for 14 hours a day all year round.

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Few vivarium-maintained juveniles exercise adequately or graze over large areas. Humidity in a vivarium for a basking species is often very low and this has been suggested to be a potential contributing factor as neonates in the wild spend a great deal of time sheltering from potential predation in areas where relative humidity is high (Meyer: personal communication 2002). Food of high quality and volume is generally provided within easy reach of the tortoises daily. Keepers seldom expose their juveniles to occasional periods where food is scarce such as would often occur during the heat of mid-summer in the wild.

In captivity, hibernation is commonly avoided during the early years, when many keepers fear their juveniles to be at risk of problems. It is also unusual for keepers to expose hatchlings to prolonged periods of adverse climate, such as a rainy season, where grazing is made difficult. Well-educated and caring keepers generally want the best for their tortoise all the time. They often fail to realise that it might benefit by the inclusion in its regime of controlled periods of limited feeding and cooler climatic conditions, including increased humidity. Similarly, a short, carefully managed hibernation often provides a break from excessive feeding and uncontrolled growth.

Accelerated growth and shell deformity of juveniles in captivity can be prevented by a combination of actions restricting growth and development to an acceptable level, but not to the extent of stressing or risking the animal.

We can see that it is important to avoid premature and accelerated growth in juvenile chelonians. This may be achieved by controlled feeding regimes, hibernation and appropriate husbandry techniques:

- Alternate day feeding and timed daily feeding can be used to limit growth.
- In the future it is anticipated that reference growth rate curves and values for optimum diet and environmental conditions for individual species will become available.
- Hibernating captive juveniles for short, closely-monitored periods prevents continuous annual growth.
- Controlled annual temperatures, humidity and photoperiod can mimic wild temperature and light cycles. High growth rates maintained throughout the whole year are undesirable.
- Providing controlled periods of growth and other periods of maintenance only (as would occur in the wild, with occasional adverse climatic conditions and seasonal changes) is probably the best way to reduce undesirable growth rates.

Vomiting and regurgitation

Frye (1991b) states that the causes of continual vomiting in reptiles are similar to those in higher vertebrates. However, reports of vomiting in chelonians are unusual, and the potential causes are varied and are often related to profound disease (e.g. animals may be in terminal stages of dehydration or renal disease). Vomiting is therefore a most unfavourable prognostic sign requiring comprehensive work-up and investigation.

Juvenile chelonians with profound worm burdens may vomit ascarids. Occasional reports suggest regurgitation and gastritis may result from cryptosporidiosis (O'Donoghue 1995; Graczyk *et al.* 1998). Vomiting was reported after intravenous injection of atipamezole (Lock *et al.* 1998) and may be associated with chemical insult to the chemoreceptor trigger zone as well as digestive tract and metabolic disease. Chelonians that have ingested poisonous plants such as daffodil and yew may vomit and regurgitate in a state of collapse. This author (SM) has also encountered two unusual cases: one case where vomiting accompanied gastric neoplasia and associated gastroduodenal intussusception, and a second animal with a large hepatic neoplasia. Both were in *Testudo* spp. (SM: personal observation).

Cachexia

Derickson (1976) and Elkan (1980) describe the fat bodies of reptiles and their role in energy provision during periods of anorexia. Belkin (1965) studied cachexia (wasting) and metabolism in the turtle *Sternotherus minor* and was able to calculate rate of weight loss and potential survival times for this species. Metabolic rate decreased in combination with decreasing calorific intake until a threshold metabolic rate was attained. Wasting then depleted intracoelomic and extracoelomic fat bodies. Bone marrow fat stores and extradural adipose tissue soon followed. Finally liver and brain were affected. Donoghue (1996) also points out that cachectic tortoises lose protein as well as adipose tissue and cachectic myopathy in the captive green turtle is described by George (1997). Loss of protein from vital organs impairs their functions and can become life threatening.

Radiographic, clinical and post mortem findings in *Testudo* spp. which have been anorectic for long periods include muscle loss, empty digestive tract and an increased area of radiolucency representing the lung fields, because of decreased visceral volume (Jackson & Cooper 1981b). Such radiographic findings are often referred to as 'empty tortoise syndrome'.

Donoghue (1996) proposes the treatment of energy deficiency in chelonians should first involve fluid and electrolyte replacement and then small but increasing levels of calories and nutrients. Water is gained first followed by fat and then protein; so early weight gains should not be regarded as tissue recovery as it is more likely that they represent alterations in hydration status.

Digestion in herbivorous chelonians is impaired at low temperatures. Where temperature provision is inadequate, food may be passed undigested. Samour *et al.* (1986) describe a wasting disease of giant tortoises in captivity in the United Kingdom and consider inadequate nutrition and temperature provision to be responsible. These authors encourage underfloor and improved heating in order to improve microbial degradation of food within the digestive tract and therefore nutrient availability. The effect of temperature on the chelonian digestive processes has already been described in the section dealing with digestive physiology.

Iatrogenic enteric disease

The term *sterile gut syndrome* is popular in lay texts and describes gut flora derangements, especially following antibiotic therapy (Highfield 1996). According to Frye (1991b), the intestinal flora of reptiles is often altered or destroyed by a course of antibiotic therapy, especially when administered orally. In order to stabilise animals affected in this way he advises feeding faeces from healthy reptiles of the same species, or using some form of probiotic:

- in the United Kingdom, Avipro® (Vetark, UK) is a probiotic preparation suited to reptiles;
- faeces can be stored before commencing any antimicrobial treatment and subsequently offered back to the same reptile by stomach tube when antibiotic therapy is stopped.

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- faeces from different reptiles of the same species may appear to be an attractive source of probiotic, however the donor animal will require assessment for parasites and viral disease.

Candida spp. form part of the normal intestinal flora of reptiles (Brabant 1966). Pathogenic overgrowth may be a result of previous antibiotic therapy producing an unfavourable bacterial flora, feeding excessively high proportions of fruit or chronic starvation. A *mycotic enteritis* in *Trachemys scripta*, *Chrysemys picta* and *Testudo hermanni* is described by Zwart & Buitelaar (1980). The authors treated the condition effectively using oral Nystatin and glucocorticoids at an arbitrary dose. Management of mycotic infections is covered in the therapeutics section of this book.

Enteritis is also common where herbivores have been nursed using cat and dog recovery diets.

Toxic plant and chemical ingestion

There are few clear reports of ingestion of toxic plants and chemicals in chelonians.

- Liver mercury concentrations were higher in tortoises with *Mycoplasma*-associated upper respiratory tract disease in a study by Jacobson *et al.* (1991).
- Tangredi & Evans (1997) suggest that the immunosuppressive effects of low level exposure to organochlorines, including chlordane and endosulfan, could be involved in the prevalence of ocular, nasal and otic infections in *Terrapene carolina carolina*.
- Burger *et al.* (1998) demonstrated that survivability of hatchling *Trachemys scripta* slider turtles was significantly decreased in response to lead administration. The righting response was significantly impaired and this was proportional to lead dose. Larger turtles coped better than smaller turtles.
- Holt *et al.* (1979) describe buttercup (*Ranunculaceae* spp.) ingestion associated with haemorrhagic gastritis, but the diagnosis was presented as a result of a lack of other, more plausible, explanations.
- Thiocyanates present in cruciferous vegetables such as mustard greens, collard greens, cabbage, bok choi, broccoli, Brussels sprouts, cauliflower, kale, mustard seed, rapeseed and turnips are proposed to be goitrogenic and capable of inducing secondary nutritional hypothyroidism, especially in Galapagos tortoises (*Chelonoidis nigra*) (Frye 1991b; Innis 1994; Donoghue 1996). However, in the few cases where this has occurred, cruciferous vegetables were fed exclusively. Cruciferous vegetables should not be avoided completely, as they are very nutritious and palatable to chelonians.
- Vegetables high in calcium oxalate or oxalic acid such as spinach, beet greens and Swiss chard are suggested to reduce calcium availability and may predispose to hypocalcaemia and metabolic bone disease and should be fed sparingly. However, this is largely a theoretical concern, and its clinical importance has not been proven. Innis (1994) argues that almost all green leafy vegetables are considered, for humans, to be high in oxalates, but that herbivorous chelonians are likely to have a higher tolerance of oxalates than humans. No clinical cases of oxalate urolithiasis have been documented in reptiles. Because of its toxic oxalate levels, rhubarb should *never* be offered (Frye 1991b; Innis 1994; Donoghue 1996).
- Few more specific accounts of poisoning by plant ingestion seem available but an exception is yew (*Taxus baccata*) ingestion.
- The author has encountered several cases of severe collapse and debility following ingestion of daffodil (*Narcissus pseudonarcissus*) leaves and flowers by *Testudo* spp. The cases have stabilised and recovered with supportive care over several months. Toxic hepatopathy, enteropathy, anaemia and paresis all appeared to occur.

Common plants anecdotally associated with toxicity in chelonians, or with toxicity in reptiles in general, are listed in Appendix B.

Coprophagia

Various species of chelonians, often those considered herbivorous, have been observed to consume dog and tortoise stools. Frye (1991b) advises that this should be discouraged by strict attention to hygiene and avoiding high stocking rates. In the wild, tortoises distribute their faeces over a wide area and encounter it uncommonly. In captivity, soiling of fixed enclosures results in a build-up of faecal parasites and facilitates horizontal transmission of pathogenic bacteria, protozoa, viruses and metazoan parasites with a direct life cycle. Similarly, an animal carrying a moderate number of well-tolerated parasitic agents, such as oxyurids, may reinfect itself and acquire a much higher parasite burden when maintained in poorly disinfected enclosures.

Foreign-body ingestion

This has also been mentioned earlier with respect to digestive physiology. Jacobson (1994) describes sand impaction in several Aldabran (*Dipsochelys elephantina*) and Galapagos tortoises (*Chelonoidis nigra*) as a result of chronic ingestion of enclosure substrate; other authors also report ingestion of substrate causing impaction. We have observed impaction in the red-eared slider (*Trachemys scripta*) following ingestion of gravel and *Terrapene* spp. and *Testudo* spp. as a result of ingesting sand. In cases presented at our surgery, female tortoises often appear willingly to ingest white material including bone and fragments of broken pottery during folliculogenesis. The management of intestinal foreign bodies is described in more detail later. We have also observed the passage of a sloughed section of intestine in a female *Testudo*. Presumably, this was the result of an intussusception following the ingestion of a peach stone the previous summer. The peach stone was passed at the same time as the sloughed intestine, and the animal made an excellent recovery.

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5 GENERAL CARE OF CHELONIANS

STUART MCARTHUR AND MICHELLE BARROWS

HOUSING

HOUSING TERRESTRIAL CHELONIANS

Outdoor and indoor enclosures

All terrestrial species are best maintained outside in large enclosures wherever the climate is suitable. Unfortunately, in Britain it is rarely suitable all year round, and indoor accommodation is normally required for at least part of the year (Figs 5.1–5.10).

In the wild, *Testudo* tortoises are often found in dry scrubland areas where they range widely, feeding on high-fibre vegetation. A small pen on a lawn is not a suitable substitute. They require large, well-drained enclosures in sunny locations, which ideally should be planted with a variety of edible plants. Many *Testudo* tortoises in Britain are maintained outside all year round and their keepers, unlike other reptile enthusiasts, are not accustomed to the idea of manipulating environmental parameters such as photoperiod, temperature and humidity in order to provide an optimal environment for their animal. Whilst some tortoises have survived for decades under these conditions, owners should be made aware that by keeping them under the influence of the British climate all year round, they are subjecting their animal to temperatures and photoperiods significantly different from those in which they evolved.

Many *Testudo* species are hibernated in the United Kingdom for five or six months of the year, and this may be twice as long as wild equivalents hibernate. On the other hand, Tunisian spur-thighed tortoises (*Furculachelys nabeulensis*) do not hibernate at all in the wild (Highfield 1996). For these reasons, all tortoise

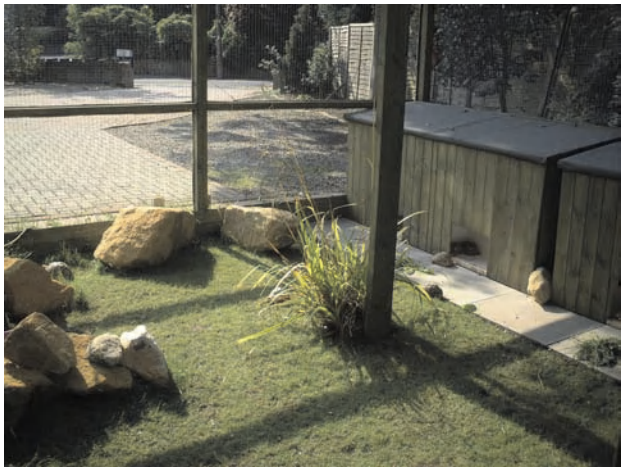

Fig. 5.1 Outdoor enclosure for a breeding colony of *Geochelone pardalis* at the author's surgery (SM).

Various shelters, hides and other forms of environmental enrichment are offered.

Fig. 5.2 Multiple basking/UVB lamps are provided within the shelters.

This is a 160W Active UVB bulb (behind) and a plain, focussed 160W basking bulb (foreground).

Fig. 5.3 At low latitudes, greenhouses are easily adapted to provide tortoise accommodation (*Testudo hermanni*).

Basking lights and ultraviolet sources complement heat provided from exposure to sunshine. Paving slabs provide thermal inertia and, by releasing their stored heat slowly, prevent excessive temperature drops overnight. (Courtesy of Frances Harcourt Brown)

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Fig. 5.4 A high-humidity chamber is constructed using large plastic storage tanks overlying heated plastic water pipes and shrouded in plastic sheeting.

Fig. 5.5 Peat and stones make an ideal substrate. Gravity-fed bathing pools are externally filtered. Basking lights and ultraviolet lights are provided.

Fig. 5.6 A purpose-constructed out-house, 'The Hilton', suited to housing larger species such as *Geochelone sulcata.* This house is insulated and provided with power which supplies underfloor heating, basking and ultraviolet lights.

A walk-through plastic sheeted door is visible to the left hand side. There is a double insulated corridor preventing excessive heat loss from the house.
(Courtesy of Graham Penney)

keepers should have available suitable indoor accommodation for use when external climatic conditions are not suitable, e.g. in the early spring after emergence from hibernation or during cold periods in the summer. Tropical or African tortoises will require indoor accommodation for most of the year but will still benefit from exposure to natural sunlight during the summer.

Fig. 5.7 Inside the 'Hilton' (Fig. 5.6). The sheeted corridor leading to the garden is obvious at the end of the house, and, to the right, basking areas are insulated with further sheeting. The central exercise area has underfloor heating and UV illumination.
(Courtesy of Graham Penney)

Fig. 5.8 An inappropriate set-up.

In the United Kingdom, many Testudo species are placed outdoors on small lawns without supplementary heating or lighting and are exposed to British weather, which is unable to sustain tortoises in a healthy state.

Where environmental provision is inadequate it may take several years before disease becomes apparent.

Tortoises are surprisingly agile and are often adept at climbing and burrowing. Males in particular may be hyperactive during the breeding season and pace the perimeter of their enclosures. Outdoor pens must therefore be well designed to prevent escape and to protect the animals from predation. Dogs, foxes, rats and birds (such as crows and seagulls) are all common predators.

Indoor accommodation may be provided in greenhouses, conservatories or polythene tunnels, or by setting up pens inside the house. Good ventilation is essential and for this reason glass tanks

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Fig. 5.9 An inappropriate set-up.
Stocking rates are excessive. Five red-eared sliders and two box turtles are housed under conditions of very poor hygiene. There is a high risk of electrocution if cleaning is attempted.

Fig. 5.10 An inappropriate set-up.
A large soft-shelled turtle is housed in this tank. There is no water filtration and heat is provided by this single halogen lamp. Exposure to bacteria is excessive and quality of life in such a tank is very poor.

and vivaria are generally unsuitable for maintaining chelonians. Open-topped pens that may be easily disinfected are recommended.

Substrate

The correct choice and depth of substrate will help in maintaining an appropriate microclimate. African hingeback tortoises (*Kinixys* spp.), box turtles (*Terrapene* spp.) and juvenile Mediterranean (*Testudo* spp.) species enjoy burrowing and should be provided with a suitable substrate for this. Substrates commonly used for chelonians include alfalfa/grass pellets, bark chippings, hemp, newspaper, Astroturf®, indoor/outdoor carpeting, peat/soil mixtures, moss and pea gravel. Sand, cat litter and crushed corn cob or walnut shells are not recommended due to the risk of ingestion and gastrointestinal impaction. Food should be provided on tiles or in dishes to reduce ingestion of substrate.

HOUSING SEMI-AQUATIC TURTLES

Semi-aquatic turtles or turtles require an area of clean water with a heated basking area. Glass tanks, plastic containers, and indoor or outdoor ponds can all be used. Some semi-aquatic turtles or turtles from temperate areas can be maintained outdoors for at least part of the year. Adult red-eared sliders (*Trachemys scripta*) or European pond turtles (*Emys orbicularis*), for instance, can be kept in enclosed ponds, although it is recommended that young turtles, those less than 10 cm in length and those with a history of health problems are maintained inside. Note that turtles are just as adept at escaping from outdoor enclosures as terrestrial tortoises.

Water

Good water quality is essential. A filtration system should be used in conjunction with regular water changes. Internal foam canister filters or under-gravel filters are usually adequate for small tank-housed specimens. Chlorinated water can be used for all aquatic chelonians, but may affect the efficiency of biological filtration systems. Where larger turtles are housed in ponds, water quality can be maintained by using an external power filter.

As a guide, water depth should be at least 1.5 times the length of the turtle's shell. Water temperature can be maintained by the use of heat pads under the tank or a thermostatically controlled water heater. Mesh covers should protect glass heaters.

Haul-out area

A haul-out area with a basking lamp should be provided for most species. For red-eared sliders (*Trachemys scripta*) the temperature at the basking site should be around 26–30°C. A sloping ramp may be needed to enable the turtle to haul itself up onto the basking area. Female turtles should also be provided with a suitable nesting area in order to encourage oviposition.

STOCKING LEVELS

It is important to avoid overcrowding, both for reasons of hygiene and because some aquatic turtles, such as soft-shelled turtles (*Trionyx* spp.) are extremely aggressive. Boyer & Boyer (1994) suggest that the combined surface area of all the occupants' carapaces should not exceed 25% of the floor's surface area.

Most chelonians are best kept in small species-specific groups. Keepers should be made aware of the dangers of introducing new animals. Few species tolerate groups of greater than eight animals, and whilst the optimal numbers for a colony of each species encountered is not available at the time of writing, this author (SM) would encourage subdivision of large colonies into closed groups of eight or less wherever possible.

Most animals live a solitary existence in the wild with males being nomadic, seeking out females for mating, and females being territorial, awaiting the arrival of the nomadic male. This is reflected in the captive behaviour of many species, where a healthy male will constantly attempt to escape its enclosure however large it may be.

Of species commonly encountered by the author, *Testudo horsfieldi* is perhaps more gregarious than most, living in groups in burrows in the wild. This animal benefits from being maintained

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in groups as opposed to in isolation. Some species, such as the Fly River turtle or pig-nosed turtle (*Carettochelys insculpta*), may show significant intra-species aggression and may need to be kept individually at most times. Keepers must closely observe groups for signs of aggression and dominance. Overcrowding should be avoided and animals of differing sizes kept apart. Introductions of new animals to established groups must be made carefully. With the high prevalence of viral disease in captive populations, even individuals isolated or quarantined for several years cannot be guaranteed as free from infectious agents.

TEMPERATURE, LIGHTING AND HUMIDITY

Correct temperature, light and humidity provision are crucial for the well-being of captive chelonians; without these, ill health is inevitable.

Sadly, the effects of inappropriate conditions are not apparent for several years, and may become so only when the animal dies! However, this author (SM) would stress that such keepers are more than balanced by the high standards and progressive techniques of the majority of competent keepers I have encountered. Inappropriate care leading to death was the fate of millions of *Testudo* species imported into Britain in the late seventies.

According to Vinter & Green (1961), approximately 240,000 tortoises (80 tons) were imported from Morocco to Great Britain in 1960 alone. Of these, only 1% survived the first year in captivity! Traditionally, these animals were placed out in our highly unsuitable cold and dark back gardens, without supplementary heat and light, all summer. Then, after struggling for all but a few weeks or months, they were routinely weighed and placed in boxes, without food or water, and chilled and dehydrated for six further months. At that time the majority of keepers knew no better. We are now generally more aware that these animals had evolved to cope with a very different existence to the conditions that prevail in the United Kingdom.

A species is both challenged and sustained by the environment in which it has evolved. If this relationship is ignored illness will often result. The animal is adapted to suit its natural habitat. Placing reptiles in completely alien environments and habitats will no doubt encourage selection of hardy animals. However, after two decades many improvements in husbandry, many of the surviving captive *Testudo* spp. in the United Kingdom have begun to breed. It is not certain that the next generation of captive-bred animals will be able to cope with ambient United Kingdom weather conditions any better than the original imported population. Just because the parents are tough does not mean that the offspring will be. We encourage all keepers to do their best to mimic the conditions that the species evolved to cope with in the wild.

Table 5.1 summarises the conditions of temperature, humidity and housing that are advised for keeping individual species of chelonian in captivity.

TEMPERATURE

Appropriate ambient temperature management is essential to the care of all behaviourally-thermoregulating exothermic reptiles in captivity. Most body processes are highly temperature dependent (Huey 1982). In reptiles, these include metabolic rate, digestion, growth, cardiovascular function, acid–base regulation, water balance, reproduction, immune function and activities such as locomotion and prey acquisition (Lillywhite 1987). This temperature-dependence has important clinical implications:

- body temperature can dramatically affect the behaviour and appearance of reptiles so it is important to examine patients at their T_s (see Table 5.2);
- antibiotic pharmacokinetics are affected by body temperature;
- the effectiveness of many drugs is temperature sensitive;
- higher body temperatures lead to more rapid recovery from anaesthetics;
- exposure to excessively high or low temperatures may hinder healing and recovery of ill chelonians, and possibly cause or exacerbate disease.

All enzymatic processes are temperature dependent. Important enzyme-controlled functions include most metabolic activities, particularly cellular delivery of energy, creation of body proteins and hormones, cell division and digestion. As a result of influences on peripheral stem cell division and bone marrow activity, even the effective functioning of the immune system is temperature dependent. Ambient temperature dictates the rate of all anabolic, digestive and homeostatic activities of reptiles.

Where inappropriate temperatures are provided, ill health will follow, albeit slowly in many cases. The provision of inappropriately low temperatures may compromise the immune system and slow anabolic processes. This is contrary to the creation of a therapeutic environment and exactly the opposite of what is desired when treating sick chelonians. Animals are likely to feed less, and reproductive behaviour and digestive efficiency will decrease.

At this author's clinic (SM), many chelonian species show significant alterations in activity and feeding when ambient temperatures are maintained above and below approximately 26°C. A *Testudo hermanni* hospitalised at 22°C may be vaguely active and responsive to stimuli but when brought to 26°C it may show increased feeding behaviour and movement. In the case of mature males, increasing temperatures above around 26°C may also reveal pacing and other sexual behaviour. Such behaviour might easily be misconstrued as distress, or distressing to the animal. In reality, however, it may go hand in hand with appropriate care and may be how the animal is able to show that temperature provision is satisfactory. A reduction in ambient temperature to reduce male sexual behaviour may also reduce anabolic healing processes.

Most terrestrial chelonians can be simply categorised into basking and non-basking species. Suitable heat provision for these animals is described later in this section, along with comments on semi-aquatic and marine species.

Terminology

The terminology used in discussion of chelonian body temperature can be confusing: safe temperature ranges, optimum temperatures, preferred temperatures, selected body temperatures, thermal neutral temperature zones, critical temperatures, preferred optimum temperature zone (POTZ), preferred body temperature (PBT) and appropriate temperature range (ATR) have all been used. Table 5.2 may help to clarify them.

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Table 5.1 Conditions for keeping common species of captive tortoises in the United Kingdom.

	Suggested temperature range for maintenance and hospitalisation (ATR) °C	Temperature exposure required temporarily during the day to ensure optimum and preferred temperature exposure is achieved (°C)	Basking hot spot of 40°C or more to be offered	Full spectrum/UVB lighting	Humidity (Low < 35% Medium 35–55% High > 55%)	Housing
Mediterranean tortoises						
Hermann's tortoise *Testudo hermanni* spur-thighed tortoises *Testudo graeca* *Testudo ibera* *Testudo whitei* *Furculachelys nabeulensis* marginated tortoise *Testudo marginata*	20–32	26–30	Yes	FSL/UVB lighting essential	medium	outdoors in large, well-drained, secure enclosures in sunny location with sheltered sleeping quarters; in spring and autumn may need warm, dry indoor area with appropriate heating and lighting
Horsfield's tortoise *Testudo horsfieldi*	20–30	25–30	Yes	FSL/UVB lighting essential	low to medium	in summer, best outdoors in well-drained enclosures in sunny location with sheltered sleeping quarters; good climbers and burrowers; intolerant of damp; in spring and autumn will need warm, dry indoor area with appropriate heating and lighting
North American semi-aquatic turtles						
red-eared turtle/ slider *Trachemys scripta elegans*	20–29 Water temperature 20°C–24°C for adults, 22°C –26°C for juveniles	24–29	Yes	FSL/UVB lighting recommended	high	provide land area with basking facilities; good water quality essential; indoor accommodation when small; adults can be kept outside in ponds during the summer
North American box turtles						
three-toed box turtle *Terrapene carolina*	21–30	24–30	limited	FSL/UVB lighting recommended; provide shady areas for retreat	high	can be maintained indoors or outdoors once acclimatised; provide plenty of cover and hide box; like to burrow in substrate; need pool for bathing; provide a few hours basking time daily
ornate box turtle *Terrapene ornata*	21–30	24–30	limited	FSL/UVB lighting recommended; provide shady areas for retreat	medium	can be maintained indoors or outdoors once acclimatised: provide plenty of cover and hide box; like to burrow in substrate; need pool for bathing; provide a few hours basking time daily

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Table 5.1 (*cont'd*)

	Suggested temperature range for maintenance and hospitalisation (ATR) °C	Temperature exposure required temporarily during the day to ensure optimum and preferred temperature exposure is achieved (°C)	Basking hot spot of 40°C or more to be offered	Full spectrum/UVB lighting	Humidity (Low < 35% Medium 35–55% High > 55%)	Housing
African hingeback tortoises						
Bell's hingeback tortoise *Kinixys belliana*	24–32	28–32	probably not	FSL probably not necessary; dislike bright lights	medium to high	warm indoor accommodation required for most of the year; provide ample cover, a hide and basking and bathing areas; bark mulch, peat or soil are suitable substrates; can be kept outdoors in well-planted enclosure with heated overnight shelter during the warmest summer weather
Geochelone species leopard tortoise *Geochelone pardalis*	20–32	26–32	yes	FSL/UVB lighting essential	low	best maintained outdoors in warmer summer months; will require shelter overnight and warm, bright and dry indoor accommodation for rest of year; needs large, well-drained grassy enclosure in sunny location
African spurred tortoise *Geochelone sulcata*	20–32	26–32	yes	FSL/UVB lighting essential	low	best maintained outdoors in warmer summer months; will require shelter overnight and warm, dry indoor accommodation for rest of year; need large, well-drained, grassy enclosure in sunny location
red foot tortoise *Geochelone carbonaria*	23–29	25–29	limited	FSL/UVB lighting recommended; provide shady areas for retreat	medium to high	large indoor pen; can be kept outside during hot summer days; provide water for bathing, ample shade and plenty of plant cover
Yellow-foot tortoise *Geochelone denticulata*	23–29	25–29	limited	FSL/UVB lighting recommended; provide shady areas for retreat	high	large indoor pen; can be kept outside during hot summer days; provide water for bathing, ample shade and plenty of plant cover
Desert tortoise *Gopherus agassizii*	20–32	26–32	yes	FSL/UVB lighting essential	low	in summer best outdoors in large, well-drained enclosures in sunny location; will need overnight shelter/burrow; in spring and autumn will need warm, dry indoor area with appropriate heating and lighting
Asian box turtles						
Malayan box turtle *Cuora amboinensis*	24–32	28–32 Water temperature of 24°C–26°C	limited	FSL/UVB lighting recommended; provide shady areas for retreat	high	require year-round indoor accommodation with equal areas of land and water for swimming; provide plenty of plant cover and basking area

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Table 5.2 Terminology associated with temperature management in reptiles (adapted from DeNardo 2002 with kind permission).

Cold-blooded vs. warm-blooded	Many reptiles have body temperatures near or even above those of some mammals. Therefore, the use of these terms in reptile medicine should be discouraged.
Poikilotherm vs. homeotherm	A poikilotherm allows its body temperature to vary dramatically, while a homeotherm maintains a relatively consistent body temperature. Many chelonians are thermally stable and show very little variation of core body temperature.
Ectotherm vs. endotherm	Ectotherms get the most of their body heat from external sources (e.g. radiant heat from the sun, conductive heat from hot surfaces), while the body temperature of endotherms is predominantly a result of internal heat production. The use of these terms is preferred over other, more commonly used but less appropriate, sets of terms. These terms can identify differences between mammals, reptiles and avian species.
Preferred body temperature (T_p) or selected body temperature (T_s)	The temperature selected by the individual when placed in an artificial thermal gradient and allowed to choose. This may vary with species, with individuals, and with the health of individuals, although healthy animals will generally select a temperature close to its optimal body temperature.
Optimal body temperature (T_o)	The temperature at which physiological performance is maximized. T_o values for various physiological functions outside of hibernation times tend to be relatively similar. However, this term presumes that the life of a chelonian over time is a steady state and a daily cycle of temperature provision and an annual cycle of temperature provision introduce an element of confusion to it. The optimal body temperature of a hibernating species of chelonian will need significant qualification in view of daily and annual cycles of behaviour.
Preferred optimal temperature zone (POTZ)	The term POTZ is intended to reflect the temperature range at which an animal functions best. It is not a very specific term and often means different things to different people. It is not clear if it is the veterinarian, the physiology of the animal, or the historical response to temperatures provided by keepers which define the 'preferred' and the 'optimum' in the POTZ of a given species. While the concept is valid, the term is considered inaccurate by many dealing with sick reptiles. In a sense, POTZ is a hybridised term and requires consideration of the optimum body temperature, the preferred body temperature, the selected body temperature and the thermal neutral zone, as described below. Animals capable of hibernation may well 'prefer' temperatures as low as 3°C at some times of year. This could even be a temperature that is optimum for a short while to a hibernating animal. Yet the same animal recovering from illness will have their recovery compromised if they are maintained too far from their optimum body temperature as described above.
Appropriate temperature range (ATR)	In this text the term ATR is used. It is intended to describe a temperature range where the animal is not hindered in its recovery from disease or surgery when hospitalised. Any ATR described will not force the animal to become catabolic, inactive or to prepare for hibernation. If maintained by a keeper, the ATR refers to the range of temperatures within which the animal can be safely maintained when not being prepared for hibernation and when recovering from disease or surgery. When maintained in a hospitalisation ward the term ATR as used by the authors incorporates the principles of optimum body temperature, preferred body temperature, selected body temperature and thermal neutral zone, as described below. This author (SM) points out that any ATR given here is intended to act as a safety guide for the care of chelonians and must still be considered a hybridised term.
Thermal neutral zone (TNZ)	This is the range of temperature around the optimal body temperature within which the animal does not attempt to alter its body temperature (i.e. the costs of altering body temperature outweigh the benefit obtained by performing at T_o).
Critical thermal minimum (CT_{min}) and critical thermal maximum (CT_{max})	The exact temperatures depend on exposure time. While traditionally both CT_{min} and CT_{max} were determined by the death of the test animals, these values are now based on the temperatures at which the righting reflex is lost. Again the CT_{min} needs careful assessment and qualification in a hibernating species of chelonian.
Biological temperature coefficient (Q_{10})	The relative change in performance over a 10°C (18°F) change in body temperature. For example, an animal that can run twice as fast at 30°C as at 20°C would have a $Q_{10} = 2$ for sprint speed. For most physiological processes, Q_{10} approximates 2.

Appropriate temperature range (ATR)

Like all reptiles, captive chelonians must be given the opportunity to regulate their body temperature through provision of a suitable gradient of temperatures within their appropriate temperature range (ATR), and by access to hides and cooler shelters in the presence of potent heat sources. Temperature ranges suitable for maintaining captive chelonians are species specific and are still poorly defined in the literature, especially for animals which are diseased or recovering from surgery. The ATR for most *Testudo* spp. is in the region of 20°C–32°C. Temperature ranges provided should be narrower for tropical than temperate species. Tropical species seem to benefit from less variable exposure to temperatures between 22°C–28°C.

It is unwise to allow chelonians fighting an infection, coping with metabolic disease or in the process of healing traumatic injuries to be exposed to temperatures below the lower end of their proposed ATR.

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Preferred body temperature (PBT)

The preferred body temperature (PBT) of a chelonian is often at or near the upper region of the ATR. For example, eating behaviour and activity increase dramatically when a *Testudo* sp. is maintained above 26°C. The same tortoise at 21°C may move around a bit but fail to feed. Basking species of tortoise should be enabled to attain their PBT by providing basking facilities for a proportion of each day and allowing the animal to move freely about a temperature gradient.

Minimum critical temperature (CT_{min})

The overnight ambient temperature of a vivarium or hospital enclosure can be allowed to fall in a daily cycle as it would in nature. The minimum temperature to which it is wise to expose a sick chelonian will be the bottom end of their presumed ATR. Data stating if sick chelonians are best maintained at a constant temperature or exposed to a daily cycle of temperatures and a temperature gradient is limited. This author (SM) tends to provide a daily cycle of heat provision within his reptile ward (see Thermoperiodicity below).

Thermal inertia

Thermal inertia is generally associated with size and means that large species cool slowly when compared to small. It is generally inversely related to the ratio of surface area to volume. Larger animals will be able to tolerate fluctuations in temperature better than small, which should be considered relatively delicate in this respect.

Hibernation temperatures

The temperatures considered to induce and maintain safe hibernation in chelonians differ between species. Not all chelonians hibernate in the wild state. Exposing non-hibernating species to abnormally low temperatures may cause health problems and will compromise recovery from surgery or disease. Most *Testudo* spp. will become inactive at temperatures below 15°C and enter a state of hibernation if maintained at 5°C–10°C. Ailing *Testudo* spp. should not be exposed to temperatures less than 18°C. This is also influenced by the rate at which the animal cools.

Maximum critical temperature (CT_{max})

Thermal burns and hyperthermia are associated with excessive heat (i.e. above CT_{max}). This is crucial with respect to immobile and debilitated animals that are unable to remove themselves from exposure to heat sources. Such animals require regular observation. Hides should be provided to mobile patients so that they can shield themselves from excessive sources of heat according to their temperature preferences.

Thermoperiodicity

The annual cycles of temperature and photoperiod are closely related. A photoperiod regime is described later in this section.

In the wild, ambient temperature will follow a daily cycle: it will be cool at night and hot in the middle of the day. Ambient temperatures will also cycle throughout the year: there will be longer cool periods in winter and longer hot periods in summer. This author (SM) advocates the use of diurnal temperature tables for the latitudes of origin, if available, in order to accurately reproduce them for captive animals. Peaker (1969) describes how under natural conditions, day length and temperature are inextricably entwined for reptiles. Ideally, temperature variations should follow any photoperiod cycle introduced, such as those described by Jones (1978), samples of which are given later. This is especially important for mature females where follicular development may be influenced by thermoperiodicity.

The enclosure should always be maintained within the animal's presumed ATR. A secondary heat source should be provided to enable 50%–60% of the photoperiod to be maintained at or marginally above the animal's T_o, and well within the animal's presumed ATR. This generally means that the animal's core body temperature is allowed to rise to a level consistent with its preferred or optimal body temperature.

The hottest period of the day for a reptile is usually the middle of the day and the afternoon. Temperatures provided during the morning and evening periods can be allowed to rise (morning) or fall (evening) gradually between the bottom end of the animal's presumed ATR and its preferred or optimal body temperature in a manner similar to the wild habitat. This results in a daily cycle of heat within the ATR.

Measuring enclosure temperature

Laser temperature monitoring devices, currently marketed for warehouse use in the food industry, are well suited to measuring basking and other temperatures within reptile enclosures. They are also very useful for measuring the surface temperature of reptiles within enclosures, and can be used intra-operatively to give a measure of core body temperature during coeliotomy procedures.

Maximum–minimum thermometers (suited to greenhouse use) (Fig. 5.11), and remote measuring digital temperature gauges

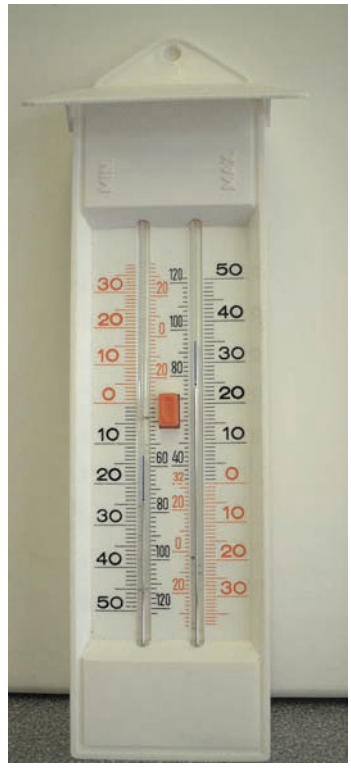

Fig. 5.11 Maximum/minimum thermometers provide a historical record of recent temperature exposure and can be used to assess hibernation temperature exposure, providing they are checked and reset on a regular basis.

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Fig. 5.12 Remote digital temperature-measuring devices, such as this hand-held laser-operated temperature gun designed for use in the food industry, are ideal for assessing both hibernation and vivarium conditions.

Fig. 5.13 This digital sensor records the temperature where the unit is as well as where the sensor is placed, and is ideal for use with both refrigerator and box hibernation techniques. It is also highly suited to monitoring the temperature of vivaria and hospital facilities.

(Fig. 5.12) are also effective tools for assessing enclosure suitability and stability, as are digital sensors which may be placed near the animals themselves (Fig. 5.13). It is important that a keeper is asked what maximum and minimum temperatures are provided, what temperature gradient is provided and what temperature is experienced in any basking area. Keepers who fail to measure temperatures frequently offer highly unsuitable temperatures to their animals without realising it. Asking them to measure and be aware of enclosure temperatures is a simple but effective way of improving the animal's environment.

Measuring temperatures within hibernacula

Temperature measurement is also crucial for the effective maintenance of hibernacula. This author (SM) uses energy-efficient and temperature-stable refrigerators to hibernate tortoises. Multiple remote measuring digital temperature devices are used. (These are available by mail order and on-line reptile or electronic equipment suppliers). Maximum and minimum alarms can be set so that a keeper is soon aware of any threats to the animal from

Fig. 5.14 Multiple digital and mercury thermometers complement each other. Digital readers should be set to alarm if temperature or humidity go outside of preset ranges. Sensor probes should be placed close to the animals.

Fig. 5.15 This digital sensor will alarm if temperatures rise to 11°C or fall to 2°C at animal level.

excessive or inadequate temperature provision (Figs 5.14–5.15). Traditional maximum minimum thermometers are used to back up such devices. Temperatures during hibernation are also discussed in other sections of this book.

Choice of heat source

Most chelonians are historically classified as basking or non-basking species and this distinction is important when setting up vivaria and hospital enclosures. Some species benefit from limited controlled basking and in such cases hides should be offered to animals to allow them to escape from excessive radiant heat. Some species are unable to tolerate direct exposure to basking heat

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sources and will become distressed and dehydrated. Such species will require very different conditions from those required for a basking animal from an arid terrestrial habitat. Large species have greater thermal inertia than small ones, and therefore maintain heat better. This is one reason why some marine and terrestrial chelonians have evolved to reach such large sizes.

Heat is often provided at the expense of humidity and the choice of heat source should take this into account. Where a desiccating heat source is used, food should be placed away from its area of strongest influence. Humidity chambers, hides, baths and misting may be necessary to maintain appropriate humidity and this is discussed later. Desiccating basking lamps should never be applied to tropical, high-humidity, photophobic species.

Heat provision

Tortoise enclosures can be safely heated in a variety of ways, with an emphasis on safety. Heating usually involves the combination of a primary background source and a secondary or variable heat source. The primary heat source generally heats the room or area containing the chelonian enclosure. (In practice, this is often a central heating system). The secondary heat source is often a ceramic heat bulb or other basking heat source or a heating pad.

Ideally all heating systems, and especially those for small enclosures, should have thermostatic control. Temperatures should be monitored by the use of digital or other thermometers, which record both maximum and minimum temperatures. Care should be taken to ensure that heat provision is always adequate and never excessive.

Where multiple animals are hospitalised in a reptile ward, it is wise to create a purpose-built heated room. Double glazing, heavy roof and wall insulation and a solid floor that is warmed on a regular basis will all maintain the background stability of the room through thermal inertia.

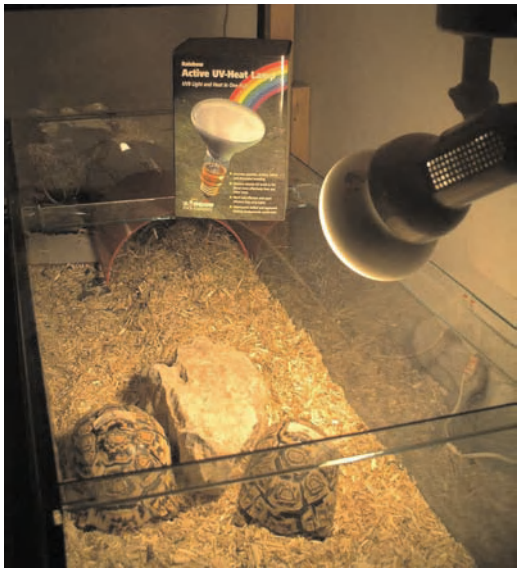

Fig 5.16 Combined basking and UVB lamps, such as this one (Active UVB®: Rainbow Rock) ensure that animals are exposed to UVB as they bask. Such combined lamps are well tolerated by most basking species.

It is essential that no plastic or glass surfaces shield the UV exposure of the animal as useful rays will be filtered.

Radiant heat

A radiant heat source is ideal for basking species. Both infrared ceramic heaters and ordinary spotlights are suitable. Many commercial companies market specific reptile basking bulbs, which also emit UVB, for this purpose (e.g. Powersun®, Zoomed and Active UVB®, Rainbow Rock) (Fig. 5.16), but domestic 40W–150W spotlight bulbs are also appropriate (Fig. 5.17–5.24).

Fig. 5.17 Mixed ultraviolet and heat lamps: Active UVB®: Rainbow Rock.

Mixed ultraviolet and heat emissions make this style of combination light ideally suited to use with basking species of chelonians.

Fig. 5.18 Mixed ultraviolet and heat lamps: Powersun®: Zoo Med.

This lamp has superseded the Active UVB from Rainbow Rock and is currently this author's (SM) preferred lamp for use in basking species.

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Fig. 5.19 UVB-emitting screw-in bulbs.
D3 Compact Reptile Lamp®: Arcadia.
This low-energy, high-output lamp emits 7% UVB and is more manageable than fluorescent tubes in most reptile enclosures. This lamp is used regularly by the author (SM) in the management of hyperparathyroidism and deranged calcium metabolism.

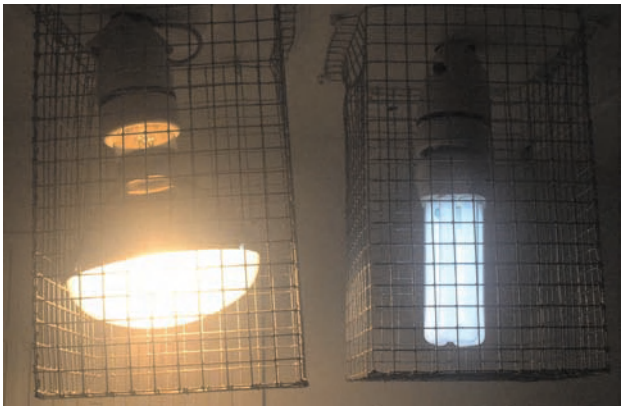

Fig. 5.20 A comparison of the differing visual output from a Powersun®: Zoo Med and a D3 Compact Reptile Lamp®: Arcadia. Where animals are suffering from metabolic bone disease (MBD) this author (SM) often uses both lamps in combination to increase the availability of vitamin D.

In basking set-ups, thermal inertia can be increased where the background or primary heat source is inadequate (e.g. at night) by the addition of stone or concrete objects such as paving slabs. The slabs will become warm during the day from exposure to radiant basking heat sources and then will give out heat slowly overnight in a fashion similar to a storage heater (Fig. 5.3). Stone arrangements can also increase the aesthetics of any enclosure.

Fig. 5.21 Heat Glo Infrared Heat Lamp®: Hagen.
This lamp is suitable for maintaining temperatures at night or for use in combination with a full-spectrum light source. Such lights are well tolerated by basking species.

Alternatively, bulbs can be switched from incandescent to ceramic or blue light at night. Insulating covers may also be beneficial at night, but basking chelonians tend to be best housed in open-topped containers during the day.

During good summer weather, basking species of temperate origin housed in greenhouses, polythene tunnels used for horticulture or conservatories may require no additional heating other than the provision of a radiant heat source for basking. Both infrared ceramic heaters and ordinary spotlights are suitable for this purpose. Supplementary heat may be beneficial and necessary at night.

Heating from below

Heating chelonians from below is fraught with potential danger and may cause ventral burns, deranged digestion and inadequate heat dissipation through the animal. Debilitated animals lying in urine or faeces and heated from below often suffer serious infections of the cloaca and plastron.

Heat pads may be useful for providing additional heat to tropical species such as the Asian box turtles (*Cuora* spp.), but they should never be placed in direct contact with the animals. Whenever radiant heat pads are used it is wise to place them against the side walls of a container as opposed to the floor, or to place tiles or other solid material on top of them so that an animal cannot suffer thermal trauma if heat input becomes unreliable.

Occasionally, underfloor or other ventral heating systems may be required to maintain ambient room temperatures for some

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Fig. 5.22 Day Glo Neodymium Daylight Lamp®: Hagen.
A commercial reptile basking lamp.
This lamp is suitable for combined use with a source of UVB (e.g. a Reptisun 5.0 tube®:Zoomed or D3 compact Reptile Lamp®: Arcadia).

Fig. 5.23 Standard 140W domestic, narrow-beam floodlight.
Whilst devoid of UVB, this type of lamp is also well tolerated as a light/heat source.

Fig. 5.24 A range of lamp bulbs suitable for day and night use and suited to nocturnal or photophobic species is a great asset to any reptile ward. Some should be dedicated basking lamps; others can be background heat sources.

species. Wherever ventral heat is applied to a chelonian it should be thermostatically controlled, protected from excessive localised heat output and regularly serviced.

Basking species

Basking species are best heated in a daily temperature cycle with variations in temperature achieved by exposure to primary and secondary heat sources above the animal. Heat sources within enclosures with basking set-ups may create localised basking areas with temperatures as high as 45°C. Here it is important that immobile animals are not exposed to such heat for unsuitable periods, and it is important that even mobile animals are provided with shade and hides.

- The carapace of basking species can be considered comparable to a 'photoreceptor'.
- The lungs of basking species can be considered comparable to a heat exchanger.
- The cardiovascular system of a basking chelonian can be considered comparable to a heat distribution system.
- The shell of basking chelonians, in combination with behavioural traits such as burrowing, acts as a source of insulation at night, increasing thermal inertia and slowing heat loss.

Basking species benefit from provision of heat from radiant sources above and around them. These may be ceramic heat sources, spotlights, infrared lamps or specific commercial reptile basking lamps. As has already been mentioned with respect to shell anatomy, they are evolved to utilise the carapace and underlying lung tissue and blood vessels as a heat receptor and exchange system.

Basking animals should not be heated directly from below. Few heat pads available to clinicians or keepers have reliable thermostatic temperature regulation and many have unpredictable hot spots. Because chelonians lack suitable pain receptors, they appear to be unable to respond to excessive heat trauma, so that unreliable heat pads can prove fatal (SM: personal observation).

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Fig. 5.25 *Testudo hermanni* hatchling.

Ventral heat mats are inappropriate heat sources. Where heat mats are used they are best placed on side walls of enclosures in order to radiate heat to the animal, rather than to heat the ventral surfaces of the animal.

The digestive tract lies relatively unprotected from heat just above the plastron within the coelomic cavity. Heating this area increases the digestive processes and can derange gut fermentation resulting in rupture of the small or large intestine and even death. This author (SM) has been presented with several hatchlings where the use of ventral heat mats has resulted in gut rupture and death and strongly advises against this form of heat provision.

Fig. 5.26 *Testudo hermanni* hatchling.

A close up of the plastron of the same animal as in Fig. 5.25 shows the effect of the coelomitis, resulting from heat-induced gut rupture, on the plastron. Often fistulas discharge foul intestinal contents. Such animals may be presented alive and appear to take a considerable time to die. This author (SM) advises urgent euthanasia of animals and advocates dorsal provision of heat to basking species wherever possible.

This author advises against the use of heat pads for basking species, unless they are placed on side walls where they act as a radiant as opposed to conductive heat sources (Figs 5.25–5.26).

Large animals of substantial size, 25 kg or more, may benefit from some form of carefully monitored underfloor heating system in their enclosure or housing (Fig. 5.7). Samour *et al.* (1986) found that inadequate floor temperature predisposed such animals to cloacal infections and reduced core body temperature to unacceptable levels, even in the presence of plentiful basking lamps.

Non-basking terrestrial species

Non-basking species tend to come from humid and dark forest floor habitats in tropical/equatorial climates. Such animals may be photophobic and can appear distressed when placed beneath bright basking lamps. Dislike of intense light and basking heat sources goes hand in hand with an affinity for high humidity and low levels of illumination.

Non-basking species, therefore, generally enjoy shaded tanks and enclosures, heated mainly by primary background heat sources and with regular misting. Secondary heat sources may be heat pads or hot water bottles placed upon heat mats. Heat mats should be placed on side walls wherever possible, or should be thermostatically controlled and with sufficient peat or similar substrate above them to prevent heat trauma to inactive animals. Placing paving slabs or large containers of hot water above heat pads will buffer animals from direct ventral exposure to excessive heat.

Coated, coloured (red/blue) or shaded bulbs may be used to provide non-basking species with background heat without excessive illumination. Spotlights should be avoided, and humidity in the area heated by lights should be monitored regularly to prevent desiccation of the environment, food or animal.

Semi-aquatic and aquatic species

Semi-aquatic and aquatic animals outside of their normal environment benefit from water heating systems and these should be caged or protected in order to prevent damage and electrocution. Some animals will enjoy basking on logs and rocks outside of the water. A haul-out area with a basking heat source and a hide should be provided wherever possible.

Hibernation temperatures

For a discussion of hibernation temperatures, see Hibernation, below.

LIGHTING

The quality of light in a vivarium or outdoor enclosure affects chelonians in a variety of physiological and behavioural ways. Reproductive synchronisation, growth cycles in juvenile animals, calcium metabolism, lipid storage mechanisms, hibernation cues and general levels of behaviour all seem sensitive to light exposure in one way or another. It is very important, therefore, that both the intensity and duration of lighting that is provided are suitable for the species in question. In addition, all captive reptiles should be offered shelter from excessive exposure to light, especially where they are unable to move of their own accord. In such circumstances regular observation is essential.

Light appears to have various beneficial effects on captive reptiles, the extent to which they are affected relating to the quality and quantity of light and the radiant heat that accompanies it. However, Jarchow (1988) points out that constant exposure to light is stressful and the natural light preference of captive reptiles should always be considered. Inappropriate light provision may predispose chelonians to disease, albeit over a considerable period of time.

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Some ground-dwelling, tropical-forest species are photophobic. These animals become highly stressed where appropriate shelter from light is not made available. Similarly, neonate and juvenile terrestrial species, which suffer greatly from bird predation in the wild, appear to become instinctively inactive, reclusive, and seek out vivarium corners and increase nocturnal behaviour when adequate hides are not provided. It is important that hides are considered in the construction and furnishing of hospital quarters for these animals.

Most chelonians maintained as pets in the United Kingdom are basking species, and the quality of light available to them has a significant effect upon their behaviour and general activity levels. People living in northern latitudes, such as the United Kingdom, where the sun's rays are spread over a large ground area in comparison to more equatorial habitats, are aware that we have, by comparison, poor-quality light. This is in addition to frequent dull, grey weather.

Such weather and dilute sunlight do not promote basking or general activity and, in this author's opinion (SM), may persuade the animal that hibernation may soon be a good option at highly inappropriate times of year. Since glass and plastic will act as an unwanted filter between an ultraviolet source and a reptile, even chelonians kept in greenhouses or conservatories should be provided with full spectrum lighting. Ultraviolet-permeable plastic is available for glasshouse building. It is however questionable if the ultraviolet wavelengths that permeate are beneficial for the animals.

Physiological mechanisms to store fat and to shut down anabolic processes may be triggered in response to low light (general visible spectrum or ultraviolet). Hepatic lipidosis may be one potential adverse consequence, hyperparathyroidism another. In the United Kingdom, if an historically garden-dwelling animal is afforded supplementary UVB lighting, dramatic and beneficial alterations in behaviour and apparent health often result. It is wise for clinicians living in areas far from the equator to encourage the owners of captive reptiles to provide supplementary lighting at least during daylight hours.

It is recommended that whenever basking, herbivorous chelonians are housed indoors they be provided with full-spectrum lighting (FSL), which emits UVB radiation. However, it must be remembered that some commercially available lights sold by pet care outlets may not provide the optimum wavelengths for chelonians, and all need to be placed very close to the animals. Unless large numbers of these lights are provided, the small amounts of beneficial UVB radiation actually absorbed by the animals may mean that they are of doubtful physiological benefit.

For this reason, all captive tortoises should be exposed to sunlight whenever the weather is warm enough. There is a significant and positive alteration in behaviour observed at this author's clinic (SM) when chelonians are examined in a high UVB environment as opposed to under traditional artificial tungsten and similar lighting. This author considers many chelonian species to be highly receptive to UVB. In this author's opinion combined basking and UVB lighting now available (e.g. Active UVB®, Rainbow Rock; Powersun®, Zoomed) are exceptionally well tolerated by *Testudo* and *Geochelone* species and offer exciting potential improvements in future captive reptile care.

Reproductive physiology and reproductive behaviour of chelonians is described in more detail elsewhere in this text. Whilst great emphasis is placed upon the role of temperature in controlling reproduction, there is also evidence that photoperiod may affect ovarian cycles, potentially through variations in serotonin/melatonin ratios in response to photoperiod (Vivien-Roels *et al.* 1979). Where captive animals are exposed to constant light and dark periods of around 12 hours, which in this author's experience (SM) is common, it is possible that breeding cues may become deranged and animals may not be able to synchronise their breeding cycles with annual seasons. This is a crucial factor for oviparous species dependent upon the environment to incubate and maintain their eggs after ovulation. It is therefore wise to consider photoperiod when keeping sexually mature chelonians in captivity. An example of a photoperiod chart suited to *Testudo* sp. is given later.

In summary:

- Use full spectrum lighting (FSL) for all basking chelonians, even during short-term hospitalisation (Reptisun 5.0® or Powersun®, ZooMed; Active UVB®, Rainbow Rock).
- Fluorescent tubes are best placed within 15–30 cm of the patient to maximise exposure to beneficial radiation.
- If weather permits, animals should be placed outdoors and allowed to bask in the sun.
- Implement an annual photoperiod cycle to reinforce light-sensitive growth, metabolism and reproductive cycles.
- UVB transmitting plastics are now available and should be utilised in enclosure construction wherever possible.

PHOTOPERIOD

Photoperiod is easily adjusted by using timers on electrical lighting circuits. It is this author's experience (SM) that alterations in timers can be made on a fortnightly or three weekly basis without detriment to the animals. By utilising an annual photoperiod schedule (Table 5.3), especially when combined with a thermoperiod schedule as discussed above, growth, activity, hormonal and reproductive patterns are naturalised. This author advocates that all sexually mature animals will benefit from an annual photoperiod, and the work of Vivien-Roels *et al.* (1979) suggests that chelonians are physiologically and homeostatically sensitive to photoperiod.

HUMIDITY

Inappropriate humidity provision predisposes chelonians to disease. There are vast differences in humidity tolerances between different chelonian species. Some very general comments on humidity requirements are given later in certain species care tables. More specific humidity advice should be sought from detailed sources, beyond this text, relating to each individual species encountered.

A combination of misting, water sources, hides and dampening of substrate will increase humidity. Removal of vivarium furniture and the use of basking and other heat sources will reduce humidity. Humidity is also affected by the percentage of plant cover and by the choice of substrate and its depth. Humidity gauges are cheap and easily placed within a vivarium and can be used very easily to monitor humidity and its variation within a vivarium environment (Figs 5.27–5.29).

Humidity tolerances generally relate to the chelonian's environment of origin. Chelonians come variously from arid terrestrial, humid terrestrial, semi-aquatic and aquatic environments. Animals

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Table 5.3 Photoperiod values for Mediterranean latitudes (Jones 1978) (Spain 36°N–42°N; Italy 38°N–45°N; Israel 32°N).

Week beginning	Hours of daylight		
	32.5°	40°	45°
Jan 1	9:53	9:12	8:38
7	9:57	9:17	8:44
13	10:02	9:24	8:52
22	10:13	9:38	9:10
28	10:21	9:49	9:23
Feb 3	10:31	10:02	9:39
12	10:46	10:23	10:04
18	10:57	10:37	10:21
24	11:09	10:52	10:39
Mar 4	11:26	11:16	11:07
10	11:38	11:32	11:26
16	11:50	11:48	11:45
25	12:09	12:11	12:14
31	12:21	12:27	12:33
Apr 6	12:32	12:43	12:51
15	12:50	13:06	13:19
21	13:01	13:20	13:36
27	13:12	13:35	13:53
May 6	13:27	13:55	14:17
12	13:36	14:07	14:32
18	13:44	14:18	14:46
27	13:54	14:32	15:03
Jun 2	14:00	14:39	15:12
8	14:04	14:45	15:19
17	14:07	14:50	15:24
23	14.08	14:50	15:25
29	14:06	14:48	15:23
Jul 8	14:02	14:42	15:15
14	13:57	14:36	15:07
20	13:51	14:27	14:57
29	13:40	14:12	14:38
Aug 4	13:31	14:00	14:24
10	13:21	13:48	14:09
19	13:06	13:27	13:45
25	12:55	13:13	13:27
31	12:44	12:58	13:10
Sept 9	12:27	12:36	12:43
15	12:15	12:20	12:24
21	12:03	12:04	12:05
30	11:45	11:41	11:37
Oct 6	11:34	11:25	11:19
12	11:22	11:10	11:00
21	11:04	10:47	10:33
27	10:53	10:32	10:15
Nov 2	10:42	10:18	9:58
11	10:27	9:58	9:33
17	10:18	9:45	9:19
23	10:10	9:34	9:06
Dec 2	10:01	9:21	8:49
8	9:56	9:15	8:42
14	9:53	9:11	8:36
23	9:51	9:09	8:34

Fig. 5.27 Correct humidity is an essential prerequisite for the effective management of many sick chelonians. This is especially true of many Asiatic species. Several methods of providing and conserving humidity are also described in the text.

Where there is a need for high humidity, this author (SM) has found ultrasonic fogging devices such as this one (Fogger, Exoterra) to be ideal add-ons to standard vivaria.

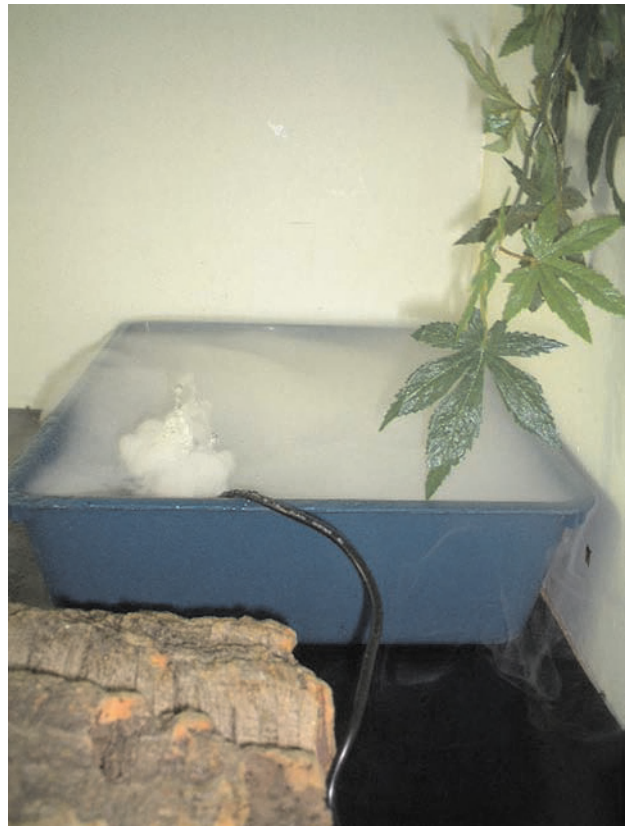

Fig. 5.28 Where humidity is required across a large vivarium, a fogging device can be placed within a water tray. This creates a layer of mist which diffuses across the floor of the vivarium. It is possible to place medication within the water tray as it acts as a simple nebuliser.

naturally living in ground conditions in rainforest habitats (such as North American box turtles, *Terrapene* spp., and Asiatic box turtles, *Cuora* spp.) have adapted to a high humidity lifestyle. They will almost certainly be highly sensitive to drier conditions, and may dehydrate significantly in situations where evaporative

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Fig. 5.29 Shy, semi-aquatic species benefit from hospitalisation within a fogged vivarium. Here plastic plants, a plastic shelter and a fogging device within the bathing water balance the effects of the basking lamp and create an ideal environment for recovery.

water losses are increased. Nitrogenous excretion patterns in high-humidity, semi-aquatic and aquatic living species are likely to be ureo-uricotelic, ureotelic or amino-ureotelic, so renal function will become significantly compromised with dehydration.

Animals living mainly in low-humidity environments (such as Mediterranean tortoises, *Testudo* spp., the African spurred tortoise *Geochelone sulcata* and the leopard tortoise, *Geochelone pardalis*) appear predisposed to ill health when maintained in unsuitably high humidity. Conditions such as respiratory and skin disease occur, especially when the animals are concurrently debilitated or where sub-optimal temperatures are provided (Table 5.4).

Fig. 5.30 Group hibernation within isolated plastic tubs in a relatively-humid class-A-efficient refrigerator, as practiced by the author (SM).

Refrigerators like this often show a marked temperature gradient from top to bottom and the upper shelves may be several degrees warmer than the lower.

The air is changed daily by opening the door and checking the animals.

Table 5.4 Some ill effects of inappropriate humidity levels.

Humidity too high	Respiratory disease Skin disease
Humidity too low	Increased evaporative losses Dehydration Hyperuricaemia and renal function compromise (especially if during hibernation)

HIBERNATION, NEONATES AND MARINE TURTLES

HIBERNATION

Figures 5.11–5.15, 5.30 and 5.31–5.35 relate to this section. Since poikilothermic reptiles cannot maintain their body temperature

Fig. 5.31 Total view of the hibernaculum during summer, when it's used as an indoor refuge.

The size is 180 × 130 × 80 cm (l/w/d).

The walls are made of impregnated chipboard insulated from the surroundings by 20 cm Styrofoam® boards. The lids are made of chipboard coated with plastic veneer. They are insulated with 16 cm Styrofoam® boards. Holes covered with wire netting allow aeration of the hibernaculum. Temperature is measured using indoor/outdoor maximum/minimum thermometers with probes. The cables of the probes are located within plastic tubing during winter to prevent tortoises becoming entangled. In winter the hibernaculum is filled over 2/3 with damp soil.

The hibernaculum has accommodated 20 *Testudo hermanni* for several years with excellent results. Weight losses during hibernation are about 1% body weight.
(Courtesy of Jean Meyer)

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Fig. 5.32 Styrofoam® boards 4 cm thick cover the interior of the hibernaculum. Spaces between the boards on two sides and the floor ensure air circulation and prevent any build-up of mould. Floorboards are covered first with a fine wire mesh to prevent soil from obstructing the air channels and second with plastic grid with rounded edges. (Courtesy of Jean Meyer)

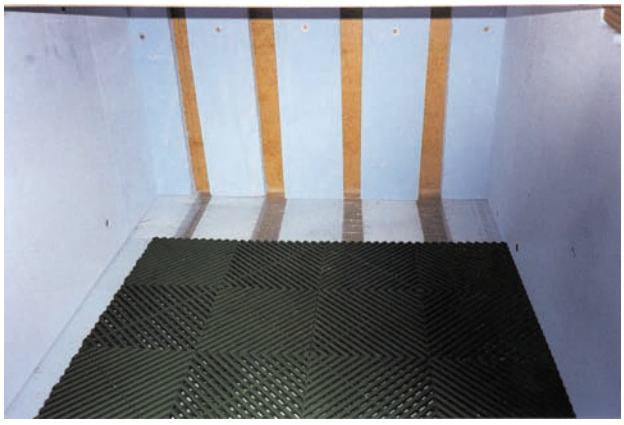

Fig. 5.33 A synthetic draining mat (plastic grid) is used to prevent soil from obstructing the lateral air channels. (Courtesy of Jean Meyer)

Fig. 5.34 The plastic grid is covered with soil.

The soil is a mixture of normal soil, 1/10 bark chips and a small amount of peat. (Peat will absorb any excessive moisture, but the amount of peat should be kept low, as it will decrease the pH of the soil, leading to skin damage in the animals.)
A heating cable is added later.
(Courtesy of Jean Meyer)

Fig. 5.35 As temperatures in winter drop to –25°C, a terrarium heating cable (100W) is fixed to the grid at the bottom. The heating coil is covered with soil and a second grid (folded back on the picture) prevents tortoises from becoming entangled and exposed to inappropriate heat.

Using a timer and a temperature probe the temperature inside the hibernaculum can be kept at a constant 5°C during winter.
(Courtesy of Jean Meyer)

independently of the ambient temperature, chelonians found in temperate areas hibernate during the cold winter months, and some chelonians from regions with very hot summers may aestivate. In the United Kingdom, most keepers prepare for hibernation after the autumn equinox. Persistent temperatures below 15°C in conjunction with decreasing day length and light quality are inducing factors.

Most hibernating species are best starved for a period of three to four weeks before entering hibernation, which in the United Kingdom, often commences at about the third week in October. However, keeping animals at normal room temperature with normal activity during hibernation preparation where food is withheld, may result in animals losing up to 5% of their body weight per week (Meyer: personal communication). Therefore, during this time temperature has to be gradually decreased. At the same time, animals should be bathed regularly in order to maximise hydration.

Anecdotally, hibernation is induced when ambient temperatures fall below 15°C. Hibernation is maintained between 2°C–9°C and temperatures below 0°C are liable to induce blindness and damage to limb extremities. Attempting to hibernate chelonians at temperatures above 10°C results in weight loss, chronic dehydration, the build up of catabolic toxins such as potassium and uric acid and exhaustion of energy reserves. Inadequate temperature provision in the post-hibernation period may result in replication of pathogens before recovery of immune system function. This effect can be amplified by the effects of an excessively long period of hibernation and depletion of white blood cell population over time. A short hibernation period with appropriate preparation, where the animal is warmed into its ATR as soon as possible, is safest.

This author (SM) uses increasing exposure to out-of-doors ambient temperature by placing animals in out-houses. The number of hours spent outside is increased over a period of three weeks and core body temperature is dropped on average by 5°C

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per week. Within three weeks, core body temperature will fall from around 26°C to around 13°C and at this point animals can be transferred to a fridge or other hibernacula which should be maintained within the temperature range of 2°C–9°C. Digital temperature alarms can be used to warn if this range is not maintained. A remote method of measuring the temperature of the hibernation chamber is advisable (Figs 5.11–5.16).

Refrigerators are both suitable and currently popular hibernation enclosures, provided the air is regularly changed (daily) and temperature control is reliable. This is the method of choice of this author (SM). Insulated boxes have been historically popular with United Kingdom keepers but leave the potential for rat trauma, frost damage and inadequate monitoring of both hibernation conditions and hibernation duration. Natural outdoors hibernation leaves room for further complications, but remains popular with a small percentage of keepers.

High humidity of the substrate (90%–95%) is important. If the substrate is dry, the tortoises lose a lot of water through respiration. This can result in exhaustion of fluid reserves early in hibernation. Good ventilation prevents building up of mould.

In the wild, in more equatorial, southern latitudes, hibernation may be weeks or months shorter than in captivity in the United Kingdom. This point should be considered when determining the duration of hibernation. Left unmanaged, most hibernating tortoises in the United Kingdom appear to awake in late March or early April and hibernate in excess of five and a half months. This may not leave enough grazing time to achieve adequate nutrition before the next hibernation. Animals may be weakened by such husbandry, failing to recover their bone marrow and other organ functions appropriately in the limited warm and sunny active time the more northern latitudes afford them. Table 5.5 gives suggested hibernation and over-wintering conditions for some of the species more commonly encountered in the United Kingdom.

Safe hibernation management

Animals can be handled carefully and checked, even in a hibernating state, for the following:

- Signs of urination indicate that further hibernation should be abandoned.
- Appropriate protection reduces the possibility of trauma (e.g. rat bites), but regular inspection is advisable where insulated boxes are placed in lofts or garages.
- Signs of activity may indicate that the animal is not being maintained at a cool enough temperature.
- Monitor ambient temperature throughout hibernation. Laser temperature measuring devices, such as that shown earlier, measure surface temperature which should correlate well with core body temperature (Fig. 5.12).
- Make regular weight checks. A hibernating tortoise should never lose more then 8%–10% of its body weight. If it does, it may be due to: too high a temperature in conjunction with activity; fluid loss due to low environmental humidity; urination.

Post-hibernation management

Upon awakening, or upon signs of imminent awakening, animals should be checked for any signs of clinical disease. All diseased animals should be presented immediately to a veterinarian. It is essential to observe and record the passage of urine as these animals are generally dehydrated. General points to consider are:

- Healthy animals should be bathed twice daily in shallow warm water encouraging drinking and voiding of urine and faeces.
- Where appropriate, competent keepers should administer tap water, slowly and gently, via a stomach tube at 1% of body weight per day (in divided doses) until multiple urination is achieved (i.e. animals are seen to have urinated more than once since awakening).
- Animals should be maintained in a vivarium with suitable temperature and light provision.
- Initially, succulent foods such as cucumber can be offered, and the diet changed back to a normal balance as soon as eating and urination are considered normal.
- Urination, appetite, activity, defecation and thirst should be carefully monitored and details recorded for around three weeks following hibernation.
- Animals not seen to have urinated or eaten within a week of hibernation require veterinary intervention, or improvements in environment.

CARE OF NEONATES

Boyer & Boyer (1994) explain that after pipping, the neonate emerges from the shell within one to four days and that the time spent resting within the broken shell often allows the absorption of the yolk sac. The yolk sac can be of a considerable size in relation to the hatchling. Mader (1996c) suggests that some hatchlings may require aseptic ligation and removal of yolksack remnants if they become traumatised or infected. Wright (1998) describes omphalectomy in a Galapagos tortoise hatchling.

After they emerge, it is advisable to maintain the hatchlings in a humid environment until the yolk sac has been absorbed and plastron and carapace folds have resolved. Highfield (1996) states that plastral folding usually straightens within 24–48 hrs. Boyer & Boyer (1994) advise a plastic food container lined with damp towels as a suitable enclosure for neonates. These authors also encourage regular soakings of up to three times a week. Within two weeks the hatchling should be feeding, often this occurs much more quickly. Highfield (1996) illustrates the use of propagators for the housing of terrestrial neonates. These are available at most gardening shops.

Boyer & Boyer (1992) describe the care of hatchling aquatic turtles and point out that protective cover or shelter should be provided in a manner that will not allow entrapment or drowning of turtles.

Hatchlings and juvenile tortoises are commonly maintained in indoor pens or vivaria and are often kept all year round under conditions of optimal heating, lighting and food availability by highly-motivated keepers. Such keepers have often bought the best vivarium, the best lights, the best heaters and the best food and do not expect their hatchlings to exert any effort in order to earn and enjoy this. Food is dropped in regularly, sometimes several times a day, close to the animal. The photoperiod and heat offered are often excessive, in the region of 14 hours a day all year with no consideration for cyclical adjustments. The keeper is often alarmed that something is wrong if a vast quantity of food isn't eaten every day.

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Table 5.5 Suggested hibernation or over-wintering protocols for commonly-kept chelonians.

	Protocol	Temperature range (°C)
Mediterranean tortoises		
Hermann's tortoise *Testudo hermanni* Spur-thighed tortoise *Testudo graeca* *Testudo ibera* *Testudo whitei* Marginated tortoise *Testudo marginata*	• Starve for 3–4 weeks before hibernation. • Hibernate in a box within a box, the two separated by an insulating material. Place in cool building or within a reliable refrigerator. • High humidity of the substrate is important, in conjunction with good ventilation to avoid build-up of mould. • Often useful to house indoors with supplemental heat and lighting at end of hibernation in order to restrict its duration.	• 5 (2–9). • Never allow exposure to sub-zero temperatures. • Monitor maximum day and minimum night temperatures throughout hibernation.
Tunisian tortoise *Furculachelys nabeulensis*	Should not be hibernated (Highfield 1996).	N/A
Horsfield's tortoise *Testudo horsfieldi*	• Often useful to house indoors with supplemental heat and lighting at start and end of hibernation in order to restrict its duration to 2–3 months. • Starve for 3 weeks before hibernation. • Hibernate in a box within a box, the two separated by an insulating material. • Place in cool building or within a reliable refrigerator.	• 2–9. • Never allow exposure to sub-zero temperatures. • Monitor maximum day and minimum night temperatures throughout hibernation.
North American semi-aquatic turtles		
Red-eared turtle/slider *Trachemys scripta elegans*	Although hardy specimens can survive mild winters outside, this is not recommended. Outdoor pond turtles should be over-wintered inside.	Varies with winter environment.
North American box turtles		
Three-toed box turtle *Terrapene carolina triunguis*	If maintained outdoors can be allowed short hibernation of 2–3 months. Set up in cool room as for *Testudo* spp. but place turtle in damp leaves, moss, peat or earth in order to maintain high humidity. Alternatively over-winter inside.	• 7 (7–16) (Boyer 1992b). • Never allow exposure to sub-zero temperatures. • Monitor maximum day and minimum night temperatures throughout hibernation.
Ornate box turtle *Terrapene ornata*	As above for *Terrapene carolina triunguis.* Note that wild turtles in southern part of range do not hibernate (Highfield 1996).	As above for *Terrapene carolina triunguis.*
African hingeback tortoises		
Bell's hingeback tortoise *Kinixys belliana*	In captivity usually kept under the same conditions year round and not hibernated. In the wild may become inactive during the winter and this seasonal change can be simulated in captivity in order to encourage breeding activity in the spring.	Chin (1996) suggests the following conditions: a decrease in day length from 13 to 11 hours and in temperature from 23°C–32°C to 18°C–20°C for a period of 8–10 weeks during the winter. Withhold food and basking facilities.
Geochelone species		
Leopard tortoise *Geochelone pardalis*	Do not hibernate.	
African spurred tortoise *Geochelone sulcata*	Do not hibernate.	
Red-foot tortoise *Geochelone carbonaria*	Do not hibernate.	
Yellow-foot tortoise *Geochelone denticulata*	Do not hibernate.	
Desert tortoise *Gopherus agassizii*	• Starve for 3–4 weeks before hibernation. • Hibernate in a box within a box, the two separated by an insulating material. • Place in cool building or within a reliable refrigerator.	• 5 (2–9). • Never allow exposure to sub-zero temperatures. • Monitor maximum day and minimum night temperatures throughout hibernation.
Asian box turtles		
Malayan box turtle *Cuora amboinensis*	Do not hibernate.	

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Obviously, this does not mimic the situation obtained in the wild, where food availability and quality may vary significantly throughout the year, and where there will be times when grazing is exhausted and poor weather inhibits activity and feeding. In addition, young, wild tortoises, like adults, may hibernate in winter and/or aestivate during very hot periods and will not be feeding or growing very much during such times.

Captive juveniles can therefore achieve abnormally, and detrimentally, fast rates of growth if hibernation/aestivation is prevented. High growth rates in the first few years of life have a strong correlation with central elevation or pyramiding of carapacial scutes. Similarly, high growth rates may result in weight in excess of that easily tolerated by pelvic bone structure, and pelvic musculature can pull in the caudal carapace if it is soft, proteinaceous and flexible and unable to resist the pull resulting from leg movements.

This author (SM), therefore, encourages a short, controlled hibernation of all juvenile hibernating species. This is achieved through temporary placement of juveniles into a cooled environment, such as a fridge, after appropriate preparation through removal of food, reduction of temperature and photoperiod. Such cooling and inactivity helps to control the rate of growth.

Metabolic bone disease/nutritional osteodystrophy is also common in captive chelonians. It is usually due to inadequate calcium and vitamin D provision, inappropriate dietary Ca:P ratio and/or lack of exposure to ultraviolet light. Correct nutritional management will avoid metabolic bone disease, accelerated growth and early maturity. For herbivorous species this involves providing a high fibre, low protein diet, as for adults, with a high calcium supplement (such as Nutrobal®, Vetark UK) and may include feeding every other day rather than daily. Species-specific growth curves for juvenile chelonians raised under conditions resulting in normal growth rates would be a useful aid to keepers and veterinary surgeons alike, but, unfortunately, such data is not yet available to us.

In warm weather, *Testudo* hatchlings can be housed outdoors with appropriate shelter and protection from predators. This allows natural grazing as well as providing the beneficial effects of natural sunlight and exercise. Juveniles instinctively fear predation from above and benefit from liberal availability of hides so that they do not feel threatened by birds and other animals. All outdoor enclosures should resist invasion from dogs and other pets.

MARINE TURTLES

Table 5.6 below gives details of marine turtles.

Table 5.6 Distribution, identification and diet of some common marine turtles (Ernst & Barbour 1989).

	Leatherback turtle *Dermochelys coriacea*	Green turtle *Chelonia mydas*	Flatback turtle *Chelonia depressa*	Loggerhead turtle *Caretta caretta*	Hawksbill turtle *Eretmochelys imbricata*	Olive Ridley turtle *Lepidochelys olivacea*	Kemp's Ridley turtle *Lepidochelys kempii*
Status	Endangered	Endangered	Endangered	Endangered	Endangered	Endangered	Near extinction
Distribution	Widest distributed species, throughout Atlantic, Pacific and Indian oceans, China Sea, Mediterranean.	Throughout Atlantic, Pacific and Indian oceans, China Sea, Mediterranean.	Western Australia.	Throughout Atlantic, Pacific and Indian oceans, China Sea, Mediterranean.	Throughout Atlantic, Pacific and Indian oceans, China Sea.	Tropical Pacific and Indian Oceans; eastern tropical Atlantic.	Western Atlantic; Nova Scotia to Mexico.
Weight range (adult female)	295–590 kg	104–177 kg	N/S	70–125 kg	78–91 kg	32–49 kg	32–49 kg
Straight carapace length (hatchling)	56–63 mm	~50 mm	56–66 mm	38–55 mm	39–50 mm	40–50 mm	38–46 mm
Straight carapace length (adult)	> 153 cm 244 cm reported	< 120 cm	up to 100 cm	70–125 cm up to 213 cm	63–94 cm	up to 71 cm	58–75 cm

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Table 5.6 (*cont'd*)

	Leatherback turtle *Dermochelys coriacea*	Green turtle *Chelonia mydas*	Flatback turtle *Chelonia depressa*	Loggerhead turtle *Caretta caretta*	Hawksbill turtle *Eretmochelys imbricata*	Olive Ridley turtle *Lepidochelys olivacea*	Kemp's Ridley turtle *Lepidochelys kempii*
Status	Endangered	Endangered	Endangered	Endangered	Endangered	Endangered	Near extinction
Identification characteristics	Lacks horny scutes; carapace covered in leathery skin; seven longitudinal carapacial keels. Some ability to thermoregulate.	Single pair of prefrontal scales on head; serrated cutting edge of lower jaw; bridge has four pairs of inframarginals that lack pores; four pairs of pleurals; four posterior scales. A surface-floating, basking chelonian.	Single pair of prefrontal scales on head; upturned marginal scutes; three posterior scales.	More than one pair of prefrontal scutes; no lateral fontanelles; three poreless inframarginals on bridge; five or more pairs of pleurals.	Two pairs of prefrontal scutes; sharp beak; four pairs of pleural scutes.	More than one pair of prefrontal scutes; bridge with four inframarginals; usually more than five pairs pleurals; 12–14 marginals.	More than one pair of prefrontal scutes; bridge with four inframarginals; only five pairs of pleurals; 12–14 marginals.
Diet in the wild	*Omnivorous* Prefers jellyfish, cnidarians and tunicates; may mistakenly ingest plastic bags; unrealistic to maintain in captivity.	*Omnivorous* Juvenile is more carnivorous than adult, which is predominantly herbivorous. Eats molluscs, sponges, jellyfish etc., green/brown/red algae, sea grasses and roots.	More carnivorous than *C. mydas*. Adults also eat sea cucumbers, invertebrates and prawns.	*Omnivorous* Adults primarily carnivorous: sponges, jellyfish, mussels, clams, oysters and plants (seaweed, turtle grass and sargassum).	*Omnivorous* Seems to prefer invertebrates. Hatchlings herbivorous but become more carnivorous with age.	*Omnivorous* Crabs, shrimp, jellyfish and plants.	*Omnivorous* Crabs, shrimp, jellyfish and plants.

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6 DIAGNOSIS

MICHELLE BARROWS, STUART MCARTHUR AND ROGER WILKINSON

A standard work-up of a clinical case will include a thorough review of the anamnesis, or case history, a complete physical examination, appropriate haematology and blood biochemistry assessments and faecal wet smear examination. Where the initial work-up suggests it to be useful, further diagnostic aids, such as radiography and ultrasonography (especially in mature females capable of reproductive activity), microscopy/cytology/microbial culture of exudates/aspirates of lesions, endoscopy and exploratory surgery may also be indicated (Table 6.1).

Protocols for the health assessment of terrestrial and semi-aquatic chelonians are also given by Jacobson (1988), Jackson & Lawton (1992), Boyer (1992b), Boyer (1992c), Boyer (1996b), Boyer (1998), Divers (1996b), Divers (1999), Jacobson *et al.* (1999a) and Berry & Christopher (2001). Whitaker & Krum (1999) describe the assessment of captive sea turtles. Mader (1999) and Walsh (1999) describe the assessment of wild sea turtles.

CLINICAL EXAMINATION

HISTORY/ANAMNESIS

The clinical history, or anamnesis, is the most important step in the assessment of most captive chelonians, unless a trauma or other critical case is presented as an emergency and urgent stabilisation measures must be employed first. Adequate time should be provided to fully cover the history. It might take longer than the physical examination. It is prudent to give a basic questionnaire to the keeper to fill in prior to arrival or whilst they are waiting. This saves time during the consultation and can be used to start the client thinking about important facts. It is worth pointing out to a client that it may not be possible to determine what is wrong without a detailed knowledge of the animal's care. This helps to explain why so many questions are asked and why the

Table 6.1 Summary of steps in the assessment of terrestrial chelonian health.

Anamnesis/history (details regarding events that have influence upon health)	It is often possible to make a presumptive diagnosis from history alone. A standard history form will ensure that important points are covered with every case. This author seldom examines a chelonian physically until the anamnesis has been reviewed. This gives time to observe the unrestrained animal, may allow distress behaviour to dissipate and chilled animals of a moderate size to warm up.
Clinical examination (observing, listening to, smelling and feeling the patient)	In a short consultation period, the complete physical examination of chelonians that retreat within their shells may not be possible. Temporary hospitalisation in a see-through vivarium may be helpful, especially where animals have been stressed, e.g. in a waiting room or during transportation or handling. It may be best to avoid handling a distressed animal and allow it to emerge in its own time instead.
Clinical pathology (investigative techniques applied to samples from the patient)	Jacobson (1988) suggests that diagnostic techniques adapted from mammalian medicine can be usefully applied to reptiles. Some reptile- and/or chelonian-specific tests, and a limited database of normal chelonian parameters, have become available. Clinical pathology investigations may include: • haematology • blood biochemistry • urinalysis • cytology • histology • serology • electron microscopy • faecal examination • microbiology • virus isolation
Diagnostic imaging techniques (visualisation of the internal structure of the patient)	Diagnostic imaging techniques include: • ultrasonography • radiography • endoscopy • computerised tomography and magnetic resonance imaging
Additional data	Where disease occurs in a group situation, additional data may become available from post-mortem examination and examination of tissues and other material from dead, or even sacrificed animals.

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physical examination is only performed after the anamnesis is discussed.

This author uses a standard history form to provoke discussion, gather information and promote bonding between veterinarian and keeper. Information and questions worthy of consideration are listed below (Table 6.2).

EXAMINATION

Examination room

It is advisable to consult in a warmed reptile room (20°–26°C). Contagion is an important issue when dealing with chelonians, so everything in the consulting room should be disposable or disinfectable. The room should be private and have suitable security to prevent patient escape. As most chelonian consults are

Table 6.2 Standard history-taking form.

Reference data
- Date
- Client identification
- Animal identification/Microchip number
- Species (common and scientific names)
- Presumed sex and age (from client)
- Assumed sex and age (from clinician)
- Is the animal kept on its own? If not give details
- Reason for presentation

Keeper/establishment
- Is this chelonian captive bred or wild caught?
- How long has it been in captivity?
- What is the duration of the current ownership?
- Are there any details of previous ownership?
- List the species managed by the keeper or establishment, and their numbers.
- How long has this keeper or establishment managed chelonians?
- What is the disease history and breeding history of chelonians at the establishment?
- Which husbandry groups is the keeper or establishment a member of?

Housing
- Is recent housing indoors, outdoors or both?
- What is the enclosure or vivarium like? (*a diagram is advisable*)

Environment
- Describe the captive thermal environment.
- How is heat provided in the captive environment?
- What are the maximum, minimum and average daytime temperatures?
- What are the maximum, minimum and average night-time temperatures?
- What is the humidity, how is it varied and how is humidity monitored?
- What lighting is provided?
- What is the photoperiod and how is it managed?
- Have any of the above been altered over the past two years?

Nutrition
- Describe the food provided. Does this vary with season?
- How is food offered and what is the frequency of feeding?
- Is any mineral or vitamin supplement offered? If so what type and what is it for?
- What provision has been made to ensure effective calcium metabolism?
- How is food prepared?
- How is food stored?
- Is food free from possible exposure to pesticides?
- Is food adequately washed to remove any pesticide residues?
- How is water provided?
- How often is water changed or filtered?

Observations
- Describe the attitude, behaviour and demeanour of the chelonian when in good health.
- Has the chelonian been displaying any abnormal behaviour? If so, for how long and is it altering?
- Describe activity and appetite.
- Has dietary preference altered recently?
- How often are faeces passed?
- What are these faeces like?
- How often is urine passed?
- What is the urine like?

Reproductive data
- Is the sex of this animal known? If so, how was it determined?
- What is its age and has it reached maturity yet?
- Has this animal bred successfully? If so, when was this?
- If this animal is female, when were eggs last laid?
- If male, has it demonstrated mating behaviour?
- Is this animal kept in isolation or as part of a group?
- When was the last contact with a chelonian of the opposite sex and has mating been observed?

Disease control
- What contact with animals of any species has this chelonian experienced?
- What methods of disease control does the keeper use?
- Has there been a quarantine program?
- How long is any quarantine program?
- What disinfectants are used?
- What does the keeper disinfect and how frequently is disinfection carried out?
- Do other animals have separate keepers?
- Is there a disinfection policy between groups or individuals?
- Have there been any health problems in this collection? If so describe them.
- How frequently are introductions made?
- When did this tortoise last meet a new chelonian?
- When was the last time this animal interacted with another?

Hibernation
- Is the animal hibernated?
- When is it put into hibernation?
- When is its hibernation over?
- What decides the duration of hibernation?
- What preparations are made for hibernation?
- What is the hibernation environment?
- How is hibernation monitored?
- What post-hibernation management is offered?

Further notes

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Table 6.3 Suggested equipment for the examination room.

examination surface, capable of being disinfected	sterile universal containers for urine and faeces
accurate scales	disinfectant solutions
see-through ruler or other length-measuring device	disposable wiping cloths and towels
dental sulcus-cleaning spike (or similar–for head extraction)	disposable gloves
cat urethral catheters (for gavage)	examination light
dog urethral catheters (for gavage)	ophthalmoscope
cotton buds	gags
microscope slides	protective gloves
needles and syringes	see-through vivaria to observe shy patients unrestrained
sharps container	history forms/consent forms/hospitalisation forms
blood collection tubes	diagnostic equipment (e.g. ultrasonography/endoscopy)
	anaesthetic equipment

likely to be 20–30 minutes long, seating should be considered for old or infirm keepers. Table 6.3 above lists suggested equipment for a chelonian consulting room.

Examination precautions

The examination protocol should minimise the risk of spreading disease (Fig. 6.1):

- always wear disposable gloves and change these between animals;
- clinicians should disinfect themselves between patients;
- disinfect all table-tops, examination implements and equipment between animals;
- treat all body secretions as potentially infectious;
- avoid examining or treating species or individuals not regularly maintained together at the same time;
- avoid handling patient notes after handling the patient, without adequate disinfection or glove removal;
- If possible, avoid placing the patient on surfaces such as the floor, where disinfection cannot easily be guaranteed between cases. Try to reserve an isolated consulting surface of an easily disinfectable material for this purpose.

Fig. 6.1 When managing chelonians from different sources it is crucial to practice a high level of disease control.

Disposable gloves and aprons, autoclavable utensils and the routine use of disinfectants on table-tops are all important aids to effective barrier nursing.

Sick chelonians are likely either to be shedding carried agents such as herpesvirus, or to be immunocompromised and more vulnerable to viral and other infections.

It is wise to screen hospitalised chelonians for faecal parasites and to utilise molecular tests for viruses, mycoplasma and other agents where possible.

Restraint

Chelonians rarely need significant restraint during examination. However, because they may show unpredictable aggression, the snapping turtles (*Chelydra* spp.) and large marine chelonians are exceptions. Most herbivorous animals pose no real danger to the clinician (Table 6.4) (Figs 6.2–6.7).

Fig. 6.2 When handling small terrestrial chelonians for jugular phlebotomy, examination of the mouth, gavage or oral medication, it is helpful for an assistant to immobilise the tortoise.

An assistant will find the animal easily stabilised if they lean their elbows on a worktop and face the operator who stands opposite them. The forelegs can be held back allowing simple handling of the head, or the use of a dental spike as illustrated elsewhere.

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Table 6.4 Restraint of marine turtles and large semi-aquatic chelonians.

Animals weighing <10 kg	Animals weighing <10 kg are easily handled with a hand on each side of the carapace. Small animals can be wrapped in towels, or placed in small, round containers for transportation, provided care is taken to prevent airway obstruction.
Medium-sized species and juveniles	Smaller animals can be held with a hand either side of the carapace, with the head pointing away from the operator. Larger animals are more comfortably held with one hand around the nuchal scute (the cranial carapace above the neck) and the other on the caudal carapace. Again, the beak should be directed away from the handler and other people.
Large species/ adults	Canvas slings or stretchers should be used for larger animals. Avoid placing oneself at risk during manoeuvring procedures–larger chelonians can inflict nasty injuries from flipper blows or bites (protective gloves are helpful).
	Chemical restraint may be necessary. Where used, the animal must not be left on its own or reintroduced into water while it is in recovery and a risk of airway obstruction or drowning remains.
General	Turtles out of water should have their plastrons rested on foam where possible, as they will no longer be supported by water. They should not be picked up by their flippers or placed on their carapaces.

Fig. 6.3 The same restraint technique described and illustrated in plate 6.2 can be used to facilitate procedures such as burring back the rhamphotheca.

Some additional points worth noting:

- Hypothermia should not be used as a method of restraint.
- Jackson & Lawton (1992) suggest whelping or sponge-holding forceps can be used to bring out the head, but this author advises that such methods should be considered a last resort and avoided wherever possible.

Fig. 6.4 Large terrestrial chelonians of 15 kg or more, such as this African spurred tortoise (*Geochelone sulcata*), can be immobilised by placing a strong upturned bucket or vessel beneath their plastrons. With nothing for their limbs to make contact with, these animals will remain where they are. Alternatively some animals may become relaxed if tipped into dorsal recumbency.

Animals of this size can give a hefty blow to the clinician if precautions are not taken. The hands shouldn't be placed into the leg outlets of the carapace as they can be severely squeezed by large animals and are hard to withdraw.

Fig. 6.5 Marine chelonians of moderate size can be restrained in a manner similar to terrestrial chelonians. In this case the animal is immobilised by placing it upon bricks and restraining its forelegs. Large animals can inflict serious trauma with their beaks.

- Placing gravid females into dorsal recumbency may result in displacement of eggs into the bladder.
- The use of a blunt dental spike is described below. When employing a spike, care should be taken to avoid trauma to the patient (Jacobson *et al.* 1999a).

Species, age and gender determination

Species

Detail on species morphology and species identification can be found earlier in this book in Chapter 1. It is advantageous to

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Fig. 6.6 In some larger species, such as this *Chelydra serpentina*, protective clothing and appropriate techniques should be considered and/or employed. Jackson & Lawton (1992) suggest that such species should be examined wearing heavy-duty gloves rather than rubber ones. Alternatively these animals can be examined in greater safety after they have bitten and locked onto an object such as a towel. The handler in this picture may be better advised to wear long sleeves.

Fig. 6.7 Where an assistant is unavailable, tape can be used to hold the forelimbs of animals such as this red-eared slider out of the handler's way. Tape can also be used to protect limbs when animals are handled whilst anaesthetised.

obtain a comprehensive guide to the identification of species with which you are likely to be presented. A detailed text will give the basic environmental requirements and nutrition of most species maintained in captivity. Ask the client what species they think they have and why. Check CITES permits if available.

Age

Some data will be available from interpretation of the duration of ownership of the keeper in relation to the growth of the chelonian during that time. This author usually records a chelonian's age as a hatchling, juvenile, adult or aged adult unless an accurate age is known. External morphological features, such as the development of secondary sex characteristics in juveniles or the angulations and lengths of nails and beaks in mature species, give clues as to life stage. Experience makes age determination simpler. Ask the client how old they think the chelonian is and why and remind them that a wild-captured specimen may have been adult at the start of any period of ownership.

Gender

Ask the client what sex they think an animal is and why they think this. A history of previous egg laying or relentless mounting behaviour may be forthcoming from the keeper. Sexual dimorphism will vary with chelonian species (see Chapters 1 and 3). Eye colour, tail size, cloaca-plastral distances, plastral concavity, body dimensions all give clues to the animal's gender, but depend upon the species in question. Hormone assays and endoscopic examination are beyond the scope of most consultations but have been employed in situations where certainty is of importance. Gender determination techniques are discussed elsewhere in this book. Ernst & Barbour (1989) and Highfield (1996) have good advice on gender determination.

Observation

Recently imported, acquired or transported individuals may take some time to settle before withdrawn behaviour can be identified either as acceptable or a sign of potential disease. Consider admitting and observing such animals over a period of several days. Warwick (1991) points out that diseased reptiles may show altered thermal preferences and that behavioural alterations tended to be towards higher temperatures during acute disease and lower temperatures during chronic disease.

The following are examples of observations worthy of recording:

- alertness;
- appetite;
- ability to prehend and swallow food;
- mobility;
- lameness;
- ability to avoid obstacles (this may be reveal impaired vision);
- visual reflexes (e.g. ability to track objects, menace response, pupillary reflex);
- signs of respiratory disease, such as abnormal respiratory movements or rate, abnormal vocalisation or respiratory noises, excessive neck extension, open-mouthed breathing (unless it occurs as part of a threat response);
- neurological abnormalities or signs (such as circling/abnormal reflexes);
- abnormal secretions and their rate of production;
- abnormal floatation in semi aquatic and marine species (this may suggest respiratory disease, intestinal obstruction or other intestinal disease, solid coelomic lesions or gas/air within the coelomic cavity);
- faeces (texture, frequency etc.);
- urine (texture, frequency etc.);
- cloacal tenesmus (this may be indicate oviposition, cloacal organ prolapse, cloacal or cystic uroliths, cloacitis, etc.).

Weighing and measuring

Assessments of weight and body size are only a small part of physical evaluation, which should also take into account patient

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observation, the history, or anamnesis, and the findings of a complete physical examination. That said, however, a record of weight and body dimensions should be part of any individual health assessment. Weight and length are conventionally measured in metric units. Length is best measured as midline carapace length (MCL) (United States) or straight carapace length (SCL) (United Kingdom). When measuring these do not include the curve of the shell (Figs 6.8–6.10).

Some values for expected weights of specific species of known carapace size are available. Where a population of presumed healthy chelonians is available, a relationship between body measurements and normal weight can be derived. Other specimens might then be compared to this population. However, *weight measurement is an ineffective gauge of health*. Weight/length

Fig. 6.8 Weighing and measuring the straight carapace length (SCL) or another body dimension has been often been used as a substitute for a physical examination.

Some ratios have validity in certain situations (e.g. screening tests for recently imported animals) but may be grossly misleading in assessing the health of individual animals.

Here digital scales and a clear ruler make the job relatively simple.

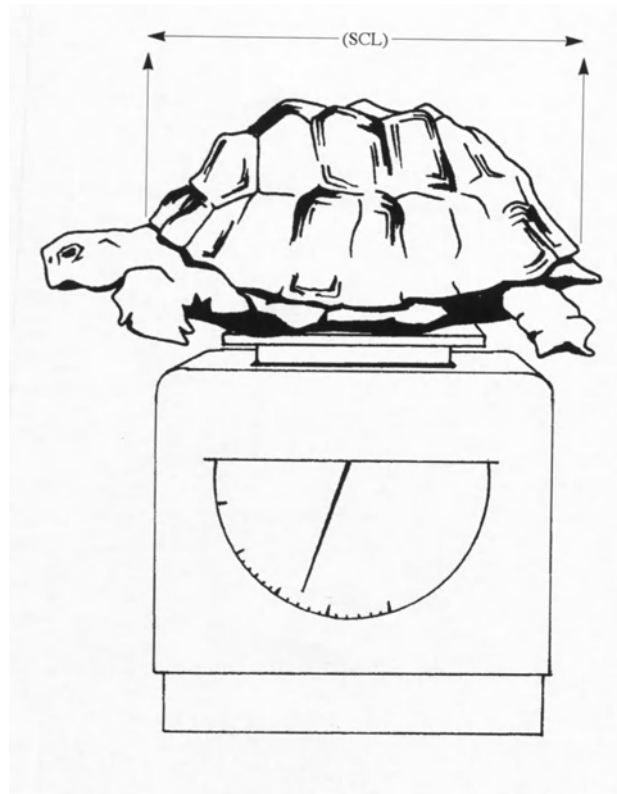

Fig. 6.9 Where straight carapace length is used, the curve of the carapace is not taken into account.

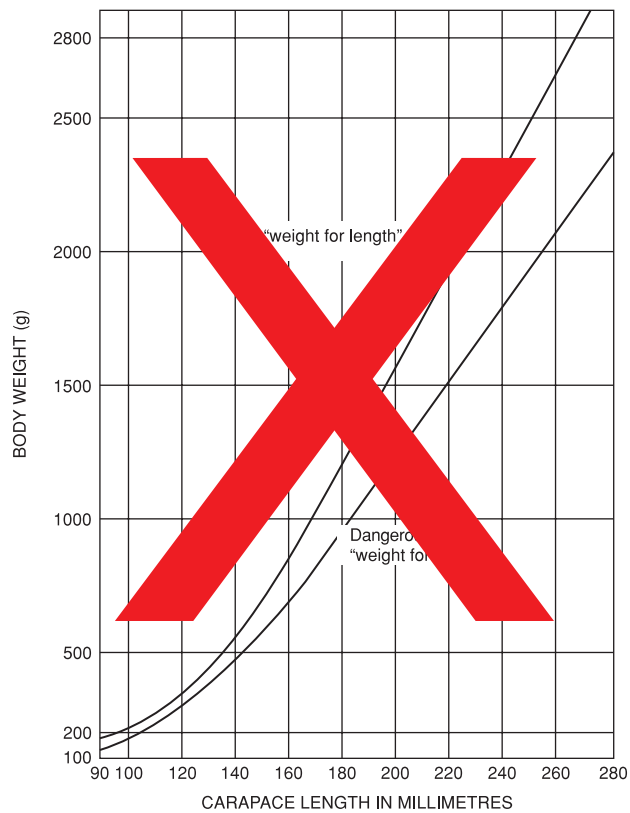

Fig. 6.10 This author (SM) does not encourage single weight and length measurements to be used as a gauge of health status.

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correlations are only meaningful with regard to health assessment if similar populations are used for a controlled comparison and alterations in health are reflected by predictable alterations in weight. McArthur (1996) was unable to differentiate sick from healthy *Testudo* spp. when weight was plotted against length following examination.

Clinical significance of weight

There are a variety of reasons why weight/length comparisons are a poor indicator of patient health (McArthur 1996; Jacobson *et al.* 1993; Jacobson *et al.* 1999a). Weight may differ with subspecies, season, diet, exercise and method of husbandry, as well as health. There are also many reasons why weights of ill animals may not deviate significantly from that of healthy populations.

A single weight measurement is of limited use in the assessment of health. In situations where weight is monitored over time, weight gain may occur with rehydration (if previously dehydrated), ingestion of food (in previously starved animals), gravidity, uroliths, intestinal foreign bodies, coelomic exudates, oedema, obesity or other illness. Anorexia/cachexia, voiding of retained fluid, or other illness may explain weight loss. Where tortoises and turtles are shy or nocturnal observation of normal activity and appetite may be restricted, weight gain or weight loss can assist in case assessment. Individual animal identification and monitoring of weight is essential when dealing with large groups, where observation of individuals may be impractical.

Some workers have suggested formulae to indicate health or illness:

- Jackson (1980b) suggested that a comparison of weight against straight carapace length might be used in the health assessment of *Testudo hermanni* and *Testudo graeca* in the peri-hibernation period. He categorised long-term captive chelonians as overweight, acceptable weight or underweight. Jackson's comparison may not apply to captive animals today as population characteristics differ from the recently imported animals he assessed (Jacobson *et al.* 1993). Jackson's data is usually presented graphically as a series of curves and can be found in other texts such as Jackson (1991).
- In North America, Donoghue (1996) describes a relationship between length and weight in 42 tortoises of nine species. She suggests the equation;

  $$\text{weight (g)} = 0.15\ \text{MCL (mm}^3\text{)}$$

  to describe healthy specimens.
- Donoghue (1996) describes further work that implies the equation:

  $$\text{weight (g)} = 0.59\ (\text{length} \times \text{width} \times \text{height})\ (\text{cm}^3) + 388$$

  was applicable to 220 healthy Californian tortoises (*Geochelone agassizii*) (Mader & Stoutenburg: unpublished data). If a tortoise has an actual bodyweight 10% less than the predicted bodyweight then Donoghue suggests nutritional intervention should be considered.
- Jacobson *et al.* (1999a) also describe weight/length assessment of desert tortoises. Weight loss of 8% or more during hibernation was taken as suggestive of a disease process.
- Recently, weight/length data has been compiled for other species including the red-eared slider (*Trachemys scripta*) (Blakey & Kirkwood 1995).

Cloacal temperature

Paradoxically, the cloacal temperature of poikilothermic reptiles provides useful information worthy of measurement during the physical examination. The thermal inertia of large reptiles makes cloacal deep-body temperature representative of the recent captive environment. This author uses the temperature probe of a pulse oximeter (Vet-Ox 4404®, Heska) to assess the deep-body temperature of animals following their transportation to the surgery. Divers (1999) points out that it is unlikely that a 10 kg leopard tortoise with a cloacal temperature of 15°C will have been removed from a 30°C vivarium in the previous hour.

Cloacal temperature is monitored when preparing reptiles for hibernation, during anaesthetic and surgical procedures and when the efficacy of heat provision is in question. Monitoring cloacal temperature is also useful in the diagnosis and management of cold-stunned turtles. Recent use of a digital, distant laser, thermal monitor device illustrated earlier has shown that surface temperature of the prefemoral or prescapular areas shows a high degree of correlation with core body temperature and can also be qualitatively used to assess temperature provision. It has also been of use in the assessment of hibernating animals.

Auscultation and percussion

An oesophageal stethoscope may allow auscultation of heart and respiratory noises, although this may not be easy in the conscious patient. Wrapping the chelonian in a dampened towel and then listening through a stethoscope placed on this towel allows auscultation of the lung fields. Be aware of movements of the limbs, which produce scratching noises, in order not to confuse them with respiratory sounds. To check for fluid in the lungs, tortoises can be tilted into a head stand during auscultation. If fluid is present, the respiratory noises increase as fluids become located over the opening of the trachea. Some soft-shelled species may be auscultated directly.

Jacobson *et al.* (1999a) describe percussion of the lung fields by tapping on the carapace. The tone of each side should be similar and a localised dull sound would suggest fluid or a solid mass. Tapping the carapace and plastron may identify abnormal or undermined scutes as they may emit a tinny sound when compared to normal scutes.

Palpation

Perhaps surprisingly, quite a bit of information may be gained from palpation:

- Fractures of the limbs may produce crepitus upon palpation.
- The shell can be palpated. Its flexibility might vary with species, age and pathology. Shells of juveniles suffering from excessive growth or metabolic bone disease have very soft shells that are easily compressed.
- The coelomic cavity can be palpated by placing a finger or thumb in each inguinal/prefemoral fossa. By rocking the chelonian gently, solid structures such as cystic calculi and calcified eggs may be felt as they bounce off the fingertips.
- The cloaca may allow digital palpation and assessment of caudal coelomic and cloacal structures. Some workers suggest that such investigation can compromise eggs present within

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gravid females and predispose to displacement of eggs into the bladder.
- Be careful when handling gravid females, as it has been proposed that placing them into dorsal recumbency may result in displacement of eggs into the bladder.

Examining the head and mouth

Bonner (2000) suggests that many chelonians retreat if the head is approached from above and in front. However a similar approach from below or behind may not be interpreted as an threat.

In most specimens of less than 10 kg, examination of the mouth is accomplished using a finger either side of the jaw to restrain the head. The other hand can be used to open the mouth. It is common practice to rest the restraining hand on the cranial carapace in order to passively prevent head retraction. Usually, it is best if the chelonian is stabilised by resting it on its caudal carapace, pointed head first towards the clinician. Alternatively, an assistant can stand opposite and present the chelonian by holding each side of the carapace. The assistant will fatigue more slowly if both elbows are rested upon a surface. This also stabilises the patient with respect to the clinician.

Shy tortoises often expose their heads if left unrestrained. The head can often be caught from behind and above if the clinician is both quick and patient – a gentle tickle of the chin or perineum may help if the chelonian refuses to play ball. Sometimes a tortoise will extend its head if a blunt instrument such as a cat urethral catheter is used to gently stimulate its tympanic scale. Many tortoises extend their forelimbs and head if tilted forward (Jacobson *et al.* 1999a).

The heads and mouths of giant tortoises can be examined by teasing them with favourite foods (e.g. an apple). Aggressive animals may gape as a threat display to the clinician. This also allows visual inspection.

In certain situations, where access to the head is not forthcoming, a probe, such as a blunt, hooked dental spike can be hooked under the upper jaw from in front and the head gently drawn forwards and out (Figs 6.11–6.23). This is easiest with an assistant gently holding back each of the front legs. Care must be taken to avoid trauma from the probe. It may be possible to fracture or pull portions of the beak off, and this should be avoided. Gentle, constant traction should be applied over a few minutes until the animal's neck muscles are exhausted. The head will then draw out slowly. It can then be immobilised by placing the thumb and index finger behind the skull. Rotating the dental probe sidewise allows the beak to be opened using the increasing width of the probe as a gag.

Fig. 6.11 Cranial examination is easiest with an assistant restraining the forelimbs from behind (*Geochelone pardalis* hatchling).

Fig. 6.12 With appropriate restraint, and where necessary, the head is presented by use of gentle restraint using a blunted dental sulcus-cleaning spike positioned behind the beak. Gentle traction allows the head to be brought forward (*Geochelone pardalis* hatchling).

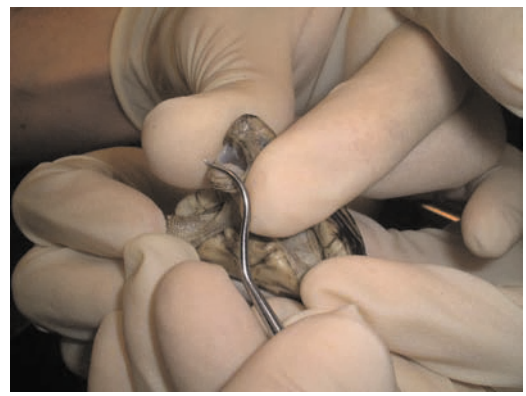

Fig. 6.13 Once presented, the head is easily controlled with the thumb and forefinger, as with this *Geochelone pardalis* hatchling.
A dental spike can be used as a gag allowing access to the buccal cavity.

Fig. 6.14 Cranial examination is easiest with an assistant restraining the forelimbs from behind (*Testudo horsfieldi*).
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Fig. 6.15 With appropriate restraint, and where necessary, the head is presented by use of gentle restraint using a blunted dental sulcus-cleaning spike positioned behind the beak. Gentle traction allows the head to be brought forward (*Testudo horsfieldi*).
(Reproduced with kind permission of British Small Animal Veterinary Association)

Fig. 6.16 Chelonians generally fatigue earlier than mammals. Once the head is gently drawn out, it is easily restrained using the thumb and forefinger placed caudal to the head, preventing its retraction.
(Reproduced with kind permission of British Small Animal Veterinary Association)

Fig. 6.17 Once the mouth has been opened using gentle pressure in the tip of the mandible, the thumb and forefinger can be used to maintain the mouth in an open presentation.
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Access to the limbs

Where species are able to retract into the confines of the shell, restraint of a swiftly caught limb often prevents the animal from full retraction. Once one limb is controlled it is often possible to gently unpeel and examine other limbs one by one. Avoid the use of levers or other instruments that may fracture or damage limbs.

Fig. 6.18 A dental sulcus-cleaning spike used as a gag, with an assistant restraining the legs. Care must be taken to avoid unnecessary trauma.

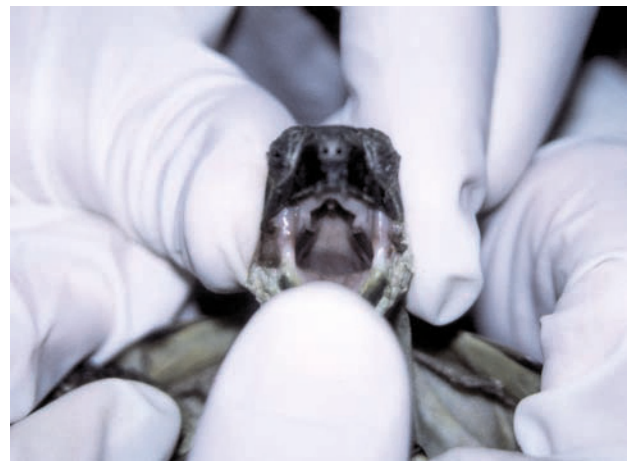

Fig. 6.19 Where a digit is used to open the mouth, remember that a chelonian will fatigue sooner than a mammal. If tension is maintained upon the jaw it will soon be possible to open the mouths of most chelonians after they give in and relax.

Care should be taken to prevent fingers being caught in the shell of species capable of plastron or carapacial closure. In some situations it may be necessary to observe the unrestrained animal during a period of hospitalisation in a see-through vivarium. In extreme cases, chemical restraint may be necessary.

Pulse oximetry

Pulse oximetry has been used in reptiles to provide a pulse rate and an estimation of arterial oxygen haemoglobin saturation (McArthur 1996; Bennett 1998; Bailey & Pablo 1998; Malley 1999: personal communication). A sheathed rectal probe suits most conscious chelonians and can be combined with a digital temperature sensor (Mader & Sala 1995; Mader 1999; Malley 1999: personal communication).

PHYSICAL EXAMINATION OF INDIVIDUALS

Shell

The shell should be examined for scute quality. Significant lesions should be recorded (possibly using a diagram). Seams between scutes give evidence of periods of growth and may suggest the

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Fig. 6.20 Once the mouth has been opened it is relatively simple to use the thumb or a finger in the angle of the jaw preventing the tortoise from closing its mouth.

Fig. 6.21 A dental spike is used to present the head of this critically ill *Geochelone pardalis* juvenile for examination.

change from wild to captive environment or alterations in husbandry/ownership.

Excessive pyramiding may relate to dietary excess of protein in combination with a high quality environment. Abnormal keratinisation, ulceration, discharges, swellings and smells should be noted. Infection and discharge typify ulcerative shell disease (USD) and a red flush of the plastron is occasionally associated with septicaemia. Poor calcification of the shell suggests metabolic bone disease (MBD).

Fig 6.22 Examination of larger animals, like this *Chelydra serpentina*, may not be possible without anaesthesia or chemical restraint.

Chemical restraint should be considered a last resort in the examination procedure. Once chemical restraint has been employed it becomes hard to interpret the significance of clinical data. It may prove appropriate for the examination of specific lesions that cannot be visualised by other methods.

Where examination proves impossible and the case warrants the effort, this author generally requests permission to hospitalise the animal for assessment/observation.

Fig. 6.23 Large animals can be offered their favourite food (this *Geochelone sulcata* likes apples!) in order to encourage them to reach out and open their mouths allowing visual inspection.

Trauma or damage may occur as a result of dog bites, injury from lawn mowers, mating trauma and exposure to fire.

Limbs

All limbs should be gently extended and their surfaces and joint flexibility examined. The legs of opposite limbs should be compared for joint size and range of movement. Joint infections and gout may be suggested by joint enlargement and swelling may be asymmetrical.

The appearance of tissues overlying the radius and ulna and tibia may be pointers to body condition. Where the bones are well padded, recent dehydration or anorexia are less likely. Where the flesh closely follows the bone contour and skin elasticity appears reduced dehydration and emaciation are possible.

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The ends of the limbs should be examined for evidence of nail overgrowth. Excessive toenail wear and foot trauma is suggestive of lameness or unsuitable confinement. Frost damage may manifest itself as oedema or swelling of the distal limbs. Abscess/fibriscess (Huchzermeyer & Cooper 2000) and tissue damage may occur at previous injection sites.

Skin

The skin should be inspected for sloughing, abnormal shedding, swellings, oedema, lesions such as abscesses and fibriscesses, ulceration, exudate and malodour. Extension of the limbs facilitates examination of the dermis of the thoracic, perineal and inguinal regions (Figs 6.24–6.25).

Decreased skin elasticity may indicate dehydration. Some viral diseases present as ulceration, papules or papillomas, especially in marine species. Bacterial and mycotic infections are common. The term septicaemic cutaneous ulcerative disease (SCUD) is often applied to severe cases, with septicaemic involvement especially in semi-aquatic species.

Excessive administration of vitamin A is associated with blistering and sloughing of the soft skin of the proximal limbs. The skin in these areas is often wet and exudes clear fluid giving lesions the appearance of pathological 'T-shirt and shorts'. Secondary infections may be present.

Blepharospasm, conjunctivitis, hyperkeratosis and abnormal shedding may relate to nutritional disease, such as vitamin A deficiency. Jaundice may present as increased yellow or green skin pigmentation, although a green colouration is normal in certain species. Increased dermal pigmentation from transient jaundice may persist into recovery.

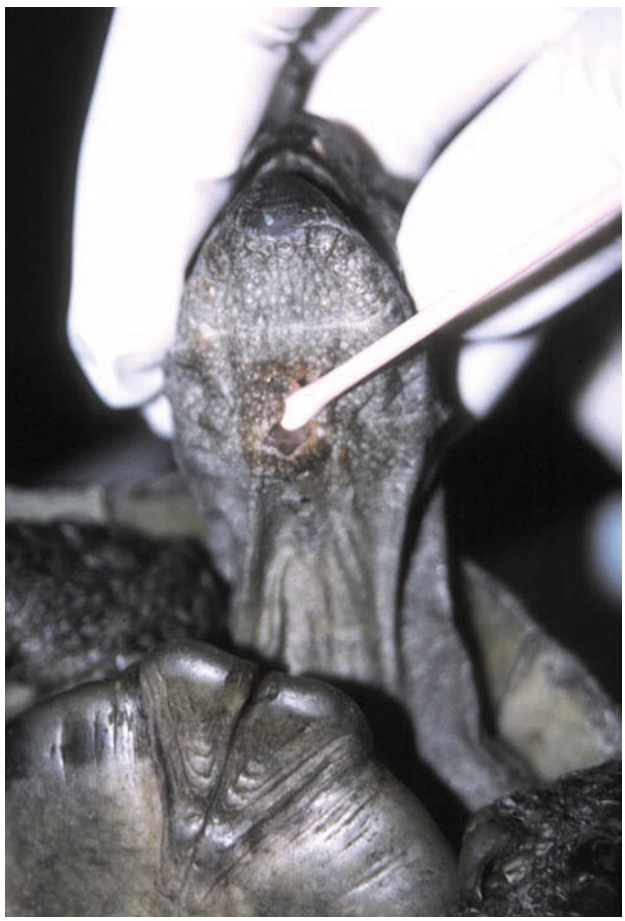

Fig. 6.24 Skin lesions and infections are amenable to bacterial, fungal and mycobacterial culture, cytology and histopathology.

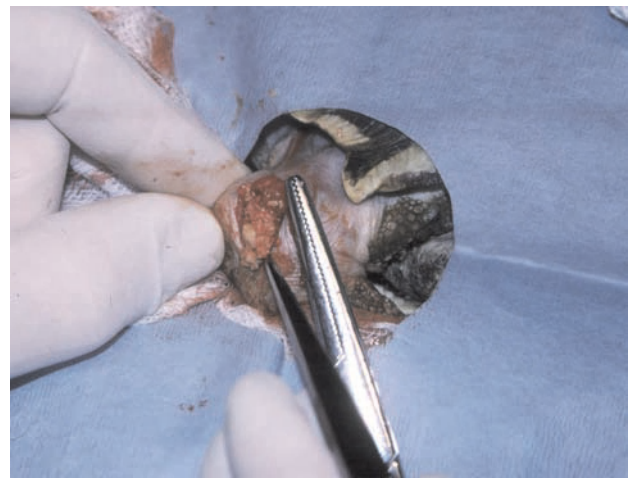

Fig. 6.25 Skin biopsies should be submitted for histopathology. Sub-epidermal tissue is less likely to be contaminated with surface organisms and so is more likely to yield the genuine pathogen on bacterial or mycobacterial culture.

The skin should be examined for parasites. Myiasis is common in warm weather, and ticks are seen in recently acquired, wild-captured specimens. Localised lesions may relate to abscess/fibriscess formation or trauma. Bacterial or mycotic granulomas are relatively common. Agents such as *Dermatophilus cheloniae* and *Mycobacterium* spp. have been identified in chelonian skin lesions.

Head and associated structures

Emaciation or dehydration may result in a decrease in tone or volume of the temporal musculature. The tympanic membrane should be examined for swelling consistent with abscess formation. The tissues surrounding the eyes, mouth, jaws, nares (nostrils) and ears should be examined.

Eye

The cornea and lens should be transparent. Ulcers, corneal plaques and pathology of the anterior chamber should be recorded. The posterior chamber and retina should be visualised using an ophthalmoscope whenever possible. Periocular tissues should be examined. In severe dehydration the eye sockets may appear to be excessively recessed.

The eyes, conjunctiva and eyelids should be inspected for evidence of swelling, abnormal discharge, corneal lesions, conjunctivitis, conjunctival hyperplasia associated with vitamin A deficiency, cataract and intraocular haemorrhage associated with frost damage, jaundice and foreign bodies.

Visual reflexes (menace and pupillary) should be assessed, although these reactions may differ between animals: some will blink and others may retract their heads. An ophthalmoscopic examination is warranted in any anorexic chelonian.

According to Boyer (1998) an ocular discharge is normal in some terrestrial species such as red-foot and yellow-foot tortoises, but abnormal in others. Many marine chelonians excrete

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unwanted ions through ocular salt glands and will demonstrate a normal ocular discharge.

Jacobson *et al.* (1999a) advise making a comparison between both eyes. According to these authors:

- unilateral enophthalmos suggests phthisis or injury;
- bilateral enophthalmos suggests dehydration or emaciation;
- unilateral exophthalmos suggests retrobulbar abscess or fibriscess, tumour or injury;
- bilateral exophthalmos suggests generalised oedema.

Beak and jaw

Fractures, overgrowth and malocclusion may prevent drinking and normal prehension of food. Instability of the jaw may occur as the result of trauma. The jaw may be soft and flexible in juvenile chelonians suffering from metabolic bone disease. Overgrowth may be associated with nutritional disease or, in my experience, supplementation with thyroxin.

Oral cavity

Examine the oral mucous membrane colour for example for pallor, cyanosis or congestion. Note the nature of any oral discharges. The mucous membranes of the oral cavity are normally pink, but stomatitis may present as erythema, haemorrhage, pallor or yellowing diphtheroid-membrane formation. Petechiation, ulceration and caseation are all consistent with stomatitis. Pharyngeal swelling may also be apparent. Consider taking an oral cytology swab, microbial culture swab or impression smear for viral, bacterial and fungal investigation (Fig. 6.26).

With the neck restrained in extension, the mouth held open and the tongue pushed up from below by finger, the glottis can be inspected. The outlets of the Eustachian tubes in the pharynx should be examined for abnormalities. Ear abscesses are often associated with pale, inspissated pus protruding into the pharynx.

If possible the choana should be visualised for evidence of inflammation, mucus or accumulation of food or foreign material. Submucosal swellings suggest gout, tumour or abscess/fibriscess formation. White inclusions in the masseter or dorsal pharyngeal muscles may suggest gout (Meyer: personal observation).

Fig. 6.26 Stomatitis.
Swabs for viral culture and herpesvirus PCR can be taken from the choana, the tongue or the oropharynx. Samples should not be taken after a recent meal as contaminants will be increased at that time.

Fig. 6.27 Upper respiratory tract disease.
Swabs for viral/mycoplasma culture and PCR can be taken from material discharged through the nares or direct from the choana. These may contain contaminants, especially if samples are harvested soon after a meal. Bacterial culture and sensitivity testing is generally unrewarding.

Fig. 6.28 Gentle digital pressure on the choana will release material for culture or molecular tests.
This can also be done with the mouth closed, massaging the intermandibular space in a craniodorsal direction.

Nares

The paired nares (nostrils) should be symmetrical and without discharge. Cutaneous erosion, softness and depigmentation often occur with chronic upper respiratory tract discharge. Boyer (1998) and Jacobson *et al.* (1999a) suggest that any nasal discharge is abnormal (Figs 6.27–6.28).

Pressing upwards in the inter-mandibular space with a digit expels material in the choana and nasal chambers through the nares allowing collection and further examination. The direct connection between the nasal chamber and oral cavity via the choana means that some conditions may affect both areas simultaneously.

Ears

The ears should be checked for abnormalities such as swelling, consistent with abscess/fibriscess.

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Cloaca

The cloaca should be examined for evidence of swelling, trauma, abnormal discharges, infection, myiasis and other parasitism. Cloacal washes may be examined microscopically. The frequency and nature of cloacal discharges and emissions should be noted. Careful digital examination may allow assessment of gravidity, colonic and cloacal tone, cystic calculi and the presence of space-occupying lesions.

Other

Is the animal microchipped?

EXAMINATION OF GROUPS

Where animals are maintained in large numbers, specific assessment of individual animals may be unrealistic. A minimum database of suitable information is an accurate record of weight variations over time for all individuals. However as described earlier, this does not equate to a health assessment. It is advisable to maximise the ability of a clinician or keeper to monitor the condition of such animals. All animals should be clearly identified, ideally with some form of semi-permanent marking or even microchip implantation, and individual records should be kept wherever possible. A useful protocol is outlined below:

- Avoid mixing animals that have previously been quarantined with others within the group.
- From any larger groups, identify animals likely to be of similar origin and genetic make up (species and sub-species), then further divide and isolate these species-specific groups into smaller units of four to six tortoises and house each within isolated and disinfectable enclosures/vivaria.
- Try to identify an ideal weight for size ratio and the ideal size for age of animal. Identify and examine closely any animal drifting outside this range.
- Number each animal and, using something like white non-toxic correction fluid, mark each animal's sex and number on its dorsal carapace, e.g. M5 or F7 etc. Alternatively, write this data on coloured stickers placed on the carapace.
- Barrier nurse each group and ensure that all groups have unique disposable or disinfectable utensils. Consider a colour-coding system for each group and all the items in contact with it.
- Try to restrict the number of keepers managing multiple groups. Where animals are divided into groups considered clinically well and clinically ill, ensure that the keepers nursing each group are different or disinfect themselves between batches.
- Impose regular periods, perhaps twice daily, when animals are observed without them being aware or handled. Follow these observations by the examination of a random sample from each group on a regular basis. If progression or regression of clinical signs suggests that the disease is changing, determine the frequency with which all animals should be intensively checked.
- Perform a regular complete physical examination of all animals in each group. This should be as often as is possible and realistic based upon staffing levels.
- Keep accurate individual records of weight, behaviour and appearance of any lesions. An accurate record of food intake, faeces output, water intake, urine output, activity etc., along with further information such as death rates, routine parasite control and any clinical signs per identified animal is ideal.
- Undertake faecal examination of samples voided from any individuals. Consider measurement of pH of urine voided from any animals during handling.
- Consider measurement of haematology and biochemistry parameters and, depending upon the likely reason for the animals' presentation, determine the frequency with which any abnormal parameters (especially those of use in assessing the prognosis), should be reassessed.
- Consider specific screening tests for pathogenic agents, e.g. herpesvirus serology and isolation.
- Keep records of deaths. Undertake post-mortem examinations of all deaths whenever possible.
- Impose realistic quarantine periods before recovered animals are allowed to mix with others, if ever.

Mixing different chelonians species within collections has been associated with outbreaks of viral or other kinds of disease. Other reptiles may also be either at risk from agents carried by chelonians or they may be sources of agents potentially infectious to chelonians. An example is *Entamoeba invadens* (Jacobson *et al.* 1983).

Several papers describe outbreaks of disease where one or more chelonian species appeared disease free whilst others about them became ill (Jacobson *et al.* 1985; Kabisch & Frost 1994; Marschang *et al.* 1997a). The potential for latent carriage of iridovirus and herpesvirus are discussed later. It would appear likely that some species innocuously carry viral agents, whilst other naïve species have a marked susceptibility to disease resulting from these agents.

Exposure of an individual to large numbers of chelonians increases the risk of acquiring new infections and should be discouraged. Where possible, we advise that chelonians should be housed in groups of less than ten, ideally four to six, in groups of the same species. All animals should be quarantined before they are mixed with others, and where specific screening tests for infectious agents are available (e.g. herpesvirus exposure, *Mycoplasma* spp. exposure) they should be encouraged. If it is necessary to congregate tortoises, remember that the incubation period of many viral diseases may be many months and animals leaving groups should be quarantined before exposure to further animals.

Wild-captured chelonians that are available for purchase may have been collected from large or multiple habitats and then forced to undergo the major stresses of mixing, diet change, environment change, chilling and transportation. Such conditions are ideal for the spread of viral diseases. Wild-caught animals like this may appear clinically normal for many months whilst incubating disease, but ultimately may prove to be carrying potentially serious pathogens. Not all animals in a dealership should be assumed to share the same disease susceptibilities or carry the same agents. Extreme caution is advised before certifying freedom from disease in such animals when they are distributed following importation. Wherever possible such animals should be screened for pathogens.

EXAMINATION OF ANIMALS IN THE WILD

Jacobson *et al.* (1999a) point out that judging health status under field conditions is not simple. First, the animals must be found

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Table 6.5 Visual inspection of marine turtles (Walsh 1999).

General condition	Weight loss will manifest in a variety of ways: • occipital ridge and caudal skull will have increased prominence; • definition of dorsal neck musculature increases with loss of surrounding fat reserves; • flippers become thinner and bonier; • carapace may appear hollow and emptier as liver and coelomic fat reserves are lost; • eyes may be sunken with weight loss or dehydration.
Lesions or disease	• External parasitism by leeches, barnacles, worms and crabs are more likely in debilitated animals. • Observe for skin lesions, such as ulceration or papillomas. • Assess all areas for wounds, injuries and lesions.
Behaviour	Behaviour assessments are different in and out of water. Turtles out of water often give the impression that they are weaker or less viable than they actually are. Out of water, normal animals will probably lift their heads when breathing and move away from stimuli. In water, observe for excessive or abnormal floatation and for coordination of flipper movements, which should be good.

and often they can only be observed at a distance. Limited access to clinicopathological tests or diagnostic imaging techniques necessitates a different approach to that which would be employed in a well-equipped clinic. Berry & Christopher (2001) elaborated protocols for the field evaluation of desert tortoise (*Gopherus agassizii*) health and disease. Judging health status of populations of wild marine species is even more challenging.

It is essential that consideration be given to the prevention of the spread of disease. Disposable gloves should be worn before handling any animal. Alternatively, the clinician or researcher should self-disinfect between handlings.

When assessing the health of a free-ranging population, one approach is to assess the area occupied by that population and then examine sample animals from typical areas. Findings from such samplings can then be extrapolated. However, potential regional influences such as terrain, habitat contamination, climate, inappropriate human interaction, predation and contact with other species of animals need to be considered. Where possible, any samples that can be stored and analysed later (e.g. cytology smears, urine, faeces, microbiology swabs and possibly blood) should be collected (Raphael *et al.* 1994a; Raphael *et al.* 1995). Appropriate clinical pathology samples and techniques are described later in this book.

An increase in the annual number of cadavers of free-ranging chelonians recovered from wild habitats, above normal levels expected, is a potential indication of recent disease. However, such deaths may occur for many reasons including infections, nutritional disease, toxicities or the results of adverse climate (Jacobson 1994).

With marine chelonians, tagging and record keeping of laying females may give a broad assessment of the overall population. It is essential that cycles and regular population variations be considered when numbers vary from year to year. Conservation techniques appropriate to marine chelonians can be found in Demetropoulos & Hadjichristophorou (1985), Lutz & Musick (1996) and Eckert *et al.* (1999).

Jacobson *et al.* (1999a) highlight the importance of accurately assessing the health of a captive chelonian by comprehensively defining criteria for reintroducing, rehoming and performing euthanasia on translocated, rescued and seized chelonians. These authors point out that where endangered animals have acquired an infectious disease for which there is no likely cure, reintroduction into a potentially naïve wild population could have disastrous consequences and must be avoided. It is therefore important that we continue to improve our ability to diagnose such conditions accurately and effectively.

MARINE TURTLES

Visual inspection

Visual assessment will allow species, sex and age determination and forms a major part of clinical examination (Table 6.5). Is the animal tagged? Unnecessary trauma such as tagging of flippers should be avoided prior to evaluation. Other procedures such as gastric lavage should also be avoided as the animal will become stressed and parameters will alter.

Common conditions

The more common pathological conditions of marine turtles are summarised in Table 6.6 below.

Table 6.6 Common conditions of marine turtles.

Captive	• prolonged forced submergence • cold stunning/hypoglycaemia • ingestion of enclosure substrate, enclosure structure or litter/debris • husbandry-related disease of illegally maintained juveniles
Free-ranging	• traumatic injuries (speedboat strikes, entanglement injuries) • gastrointestinal tract obstruction (bags, fishing hooks, other debris) • starvation leading to emaciation • cold stunning/hypoglycaemia • petrol/oil toxicity • unexplained disease syndromes and outbreaks • parasitism • fibropapillomatosis

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Criteria for release, treatment or euthanasia

Following immediate visual and physical examination in the field, some animals, such as those experiencing entanglement without significant wound or hypoglycaemic complication, may be best released immediately. Animal-side assessments, such as blood glucose measurement, can be performed with minimal complication and distress. Again, if such checks are reassuring, some animals will qualify for immediate release.

Where transportation and further complicated diagnosis or treatments are necessary, viability should be assessed maturely from the start. Where appropriate care and rehabilitation are impractical, or lesions are particularly severe, euthanasia must be considered to be a reasonable option. However, survival of cases such as that treated by Nutter *et al.* (2000), where cloacal oviductal prolapse required unilateral salpingectomy, demonstrate that even severely-compromised animals can be returned to the wild and subsequently breed. The decision for or against euthanasia is not simple.

Animals likely to harbour significant viral and other infectious agents should not be knowingly released into immunologically naïve rookeries. Formulating a realistic future care plan for rehabilitated but likely infectious individuals is not easy and these animals may be best maintained in captivity away from non-infected animals or even euthanased until the implications of the disease they carry are better understood.

Diagnostic investigations

Clinical pathology

'Normal' parameters are poorly defined, but various suggestions are given elsewhere in the clinical pathology section of this book. Blood is usually taken from the dorsal cervical sinus of the neck as described elsewhere in diagnostic techniques. The measurement techniques chosen will have significance and the use of appropriate laboratories and methods are important. Air-dried fixed smears and heparinised/heparin gel samples are appropriate.

Blood glucose is an important parameter and is easy to assess at the animal's side. Normal values are given as 3.3–6.7 mmol/l (60–120 mg/dl). The I-stat® (Heska) hand-held laboratory unit allows assessment of useful critical-care panels at the animal's side.

Diagnostic imaging

Radiography is of great help in the assessment of animals with buoyancy disorders, carapacial and head trauma, suspected pneumonia, gastrointestinal obstruction and visceral involvement in fibropapillomatosis. Endoscopy, although complicated by oesophageal papilla, may assist in the removal of oesophageal and gastric foreign bodies such as plastic bags. However, removal of fishing gear that includes hooks may be too ambitious using endoscopes. Ultrasonography is useful for reproductive assessment of mature females.

Infectious diseases

Table 6.7 lists some of the infectious diseases of marine turtles, classified according to pathogen.

Table 6.7 Some types of infectious diseases of marine turtles and their causative agents.

Bacterial and chlamydial diseases	• stomatitis complex • mycobacterial infections • dermal infections • bacterial encephalitis • chlamydial infections
Mycotic diseases	• dermal • systemic
Viral diseases	• grey-patch disease • lung eye trachea disease • fibropapillomatosis
Parasitic disease	ectoparasites: • leeches • excessive presence of barnacles endoparasites: • protozoans • helminths (spirorchiasis)

DIAGNOSTIC TECHNIQUES

POST-MORTEM EXAMINATION

Post-mortem examination of chelonians can provide the clinician with valuable information and plays a vital role in the diagnosis and prevention of disease. It is of particular importance in the group situation where early identification of disease in one or more individuals can help to prevent or manage the disease in others. Even where the cause of death is already known, post-mortem examination can yield new information and help to increase the database of knowledge for the particular species. In the zoo world, the importance of carrying out post-mortems and retaining material in order to form reference collections has been recognised in recent years (Cooper 1992). These specimens allow the retrospective investigation of disease as well as other types of research and are particularly important where endangered species such as the ploughshare tortoise (*Geochelone* or *Asterochelys yniphora*) are concerned.

Only by carrying out regular and methodical post-mortems will the clinician learn the appearance of normal tissues in a given species and be able to identify subtle changes in those from diseased specimens. The ideal post-mortem (McNamara 1999) consists of:

- a thorough evaluation of all organs;
- fixation in 10% formalin of samples from all organs;
- freezing of tissues at –70°C for possible viral isolation or toxicological or nutritional evaluation;
- bacterial and fungal cultures;
- documentation of lesions with photography;
- cytology on both normal and abnormal tissues.

In addition, post-mortem radiographs can prove useful both for evaluating the subject and to provide a species reference library for the clinician to consult in the future.

In general practice however, constraints such as time and cost to the client are likely to result in a less comprehensive examination. Where a specific diagnosis is suspected, the post-mortem

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may simply involve the harvesting of targeted organs for further examination. The use of laboratory techniques in combination with the post-mortem examination of reptiles is discussed by Cooper (2000). For more detailed discussion of reptilian microbiology and viral diagnostics see Cooper (2000) and Jacobson (2000) respectively.

Ideally the post-mortem examination should be carried out immediately after death. For this reason it might be worth sacrificing one or more of a group of sick animals and carrying out euthanasia in order to obtain specimens with the maximum diagnostic value. If not fresh, the carcase should be kept chilled until the examination can take place. Cooper (1992) states that a carcase can be kept for up to 96 hours at normal refrigerator temperature before other methods of preservation need to be considered.

Equipment and protocol

In order to carry out a post-mortem on a chelonian, the following pieces of equipment may be needed:

- scales;
- tape-measure;
- instruments including scissors, rat-toothed forceps, a scalpel and bone forceps, a saw or a modellers drill, such the Dremmel® Multi 10 000–37 000;
- sterile swabs for bacteriological, fungal or viral culture;
- syringes and needles;
- microscope slides;
- sterile containers for tissue samples;
- 10% neutral buffered formalin in screw-top containers.

The importance of a methodical approach to the post-mortem cannot be overemphasised (Table 6.8). If the organs are examined in a standard sequence and findings recorded accurately the clinician will gain the maximum benefit from the procedure. Refer to Thompson (1932), Davies (1981) and Boyer & Boyer (1996) for a discussion of normal reptilian anatomy.

Note that while the above approach is the one most often used for the post-mortem examination of chelonians, with the animal in dorsal recumbency, organs that are normally suspended from the dorsal wall will not assume their normal anatomical positions. For this reason saggital sections may reveal more useful information in some circumstances by demonstrating a more realistic view of the relationship between the lungs, bladder and other organs.

Several surveys of disease in captive terrestrial, semi-aquatic and marine chelonians review post-mortem findings and examples are given below. These may help identify the potential prevalence and spectrum of diseases in captive and wild specimens:

- Hunt (1957) describes the post-mortem findings in 200 captive chelonian deaths at London Zoo;
- Keymer (1978a) reviews the post-mortem findings of 144 United Kingdom captive terrestrial chelonians;
- A similar study by Keymer (1978b) reviews the post-mortem findings of 122 United Kingdom captive aquatic chelonians;
- Rosskopf (1981) reviews the findings of post-mortem examinations of 84 *Gopherus agassizii* and 2 *Gopherus berlandieri*;
- Recklies (1989) gives a good survey of chelonian diseases;
- Dollinger *et al.* (1997) summarises the husbandry and pathology of tortoises in three Swiss zoos.

A post mortem protocol for marine turtles is given by Campbell (1996). Analyses of bodies of marine turtles washed up on United Kingdom beaches gives some insight as to causes of mortality in free-ranging turtles (Godley *et al.* 1998).

PRACTICAL CLINICAL PATHOLOGY

For the ease of the reader, we first provide short notes of clinical techniques that a clinician may wish to refer to quickly during the physical examination. An in-depth guide to chelonian clinical pathology techniques follows, making it possible for clinicians to explore the subject in more depth at their leisure. Some material is duplicated in both sections, but we point out that this is to help the clinician as much as possible. Where possible, illustrations of blood sampling and other techniques are included.

Blood testing

Preparing blood smears

All samples for haematology should be accompanied by dried smears from fresh blood. Make ten and keep the best three. The aim is to produce a thin smear, the edges of which do not extend to the edge of the slide and which has a 'feathered' margin. Big cells such as monocytes and heterophils are concentrated at the margins of the smear. To avoid under-representation, the edges of the smear must be examinable. Gentle use of a hair dryer is recommended to achieve rapid drying with good cell preservation.

Place a small drop of fresh blood at one end of a clean slide (grease will make the smear irregular). Draw a second slide (preferably with smooth polished edges to avoid cell damage), angled at 45°, back onto it and leave there just long enough for the drop to spread along $^{2}/_{3}$ of its width. Then, in one rapid movement, drag out a smear.

Staining blood smears

Rapid stains (particularly Rapi-Diff II®, Diachem International) are quite acceptable although professional laboratories will have more time-consuming alternatives. Avoid stain precipitates by filtering or changing stain regularly.

Identification of blood cells

No-one can currently identify with complete confidence every cell in a given reptile smear. In particular the identity and identification of azurophils is an evolving area. Heterophils can be difficult to tell from eosinophils, especially if degranulated. They may be lumped together as acidophils.

- *Erythrocytes*: ellipsoid cells with a central oval nucleus and pink cytoplasm (Rapi-Diff II®, Diachem International). By virtue of their nuclei these are versatile, active cells. Mitoses, cytoplasmic vacuolation or haemoparasites are not uncommon. Immature red cells, which are commonly seen, are of variable size and rounder, with rounder, centrally-located nuclei and blue cytoplasm.
- *Thrombocytes*: also ellipsoid with nuclei. Differentiated from erythrocytes by pale blue cytoplasm and dark, concentrated, nuclear chromatin. May have a linear 'rift' along the nucleus.
- *Lymphocytes*: similar to mammalian counterpart. Small cells with eccentric nucleus and pale blue cytoplasm which may contain fine granules.

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Table 6.8 Suggested post-mortem protocol.

History: Read the history and examine clinical notes and the results of diagnostic imaging procedures or laboratory investigations. Note the age, sex, and species along with any identification details such as accession or microchip numbers. Weigh the animal and measure length of carapace and length of plastron. Take a whole body radiograph.

External examination: Assess body condition and examine the external surfaces of the body for lesions and abnormalities. Note abnormal shell shape, such as pyramiding, consistency and colour and examine any loose or discoloured scutes. Look for discharge from the cloaca, eyes, external nares or mouth. Note any swelling of the tympanic membranes.

Examine the skin for evidence of ulceration, erythema, swelling or discharge. Remove any ectoparasites for identification. Pull out the limbs and note any swelling, muscle atrophy or excessive toenail wear. Check that normal movement of joints is possible. Examine the beak for malocclusion or damage and open the mouth to examine the buccal cavity, tongue, glottis and oropharynx. Mucous membranes may be pale in normal animals following death. With some diseases they might also appear jaundiced or congested.

Note any areas of haemorrhage or ulceration and the presence of caseous or mucoid exudates. Take impression smears of any lesions for cytology and swab any exudates for bacteriology.

Place the chelonian in dorsal recumbency and obtain access to the internal viscera by cutting or sawing around the edges of the plastron followed by blunt dissection of the underlying membranes and musculature (Fig. 6.29). In larger specimens, a lever may be useful to prise open the plastron. Pull the forelimbs cranially to expose as much of the coelom as possible and incise the skin and musculature of the neck to expose the trachea and oesophagus.

Macroscopic evaluation of the coelom and viscera (Figs 6.30–6.32): Note the general appearance and relationship of the organs, the presence of any coelomic fluid and any thickening of the coelomic membrane. Note the presence or absence of fat reserves. Take swabs of any fluid present for cytology and bacteriology.

Liver: The liver is large and divided into two lobes (Figs 6.33–6.35). It should be smooth, firm and homogeneous in texture. Melanin deposits are normal in some species. Hepatic lipidosis results in an enlarged pale liver which may float when placed in water or in a formalin container. A gall bladder is present within the right lobe. If the gall bladder has ruptured, the liver and other viscera will be stained with green bile. Examine the surface of the liver and make several cuts into each lobe. Remove sections for fixation in formalin and freezing, taking care not to touch adjacent organs. Impression smears can also be made for cytology after blotting the cut surface on paper towel in order to remove excessive blood.

Heart: The three-chambered heart lies in the midline, caudal to the pectoral girdle, and the pericardial sac is continuous with the coelomic membrane. Note the presence of pericardial fluid, urate deposits or thickening of the pericardial sac. Examine the heart and note any differences in size or shape between the thin-walled atria. If septicaemia is suspected, take some blood from the heart for culture. Examine the blood vessels leaving the heart before removing it, along with the liver.

Thyroid: the thyroid gland lies in the midline cranial to the heart and is usually oval in shape.

Alimentary tract: note the external appearance of the gastrointestinal tract and reflect the small intestine to expose the large intestine. Remove the gastrointestinal tract along with the spleen and pancreas by cutting the oesophagus and rectum and breaking down the various adhesions and any attachments to the coelomic membrane. If an oesophagostomy had been performed, or oesophagostomy tube had been placed check the entry site for evidence of abscess/fibriscessation.

Follow the oesophagus towards the thicker-walled stomach and note the presence of food in the stomach, and any gaseous or fluid distension of the intestines. Take sterile samples of intestinal contents for bacteriology. Examine the oval-shaped spleen and the normally pale, elongated pancreas which are attached to the duodenum. A caecum may be present where the small intestine joins the large intestine. After external examination of the gastrointestinal tract, open it up, examine the mucosal surfaces and look for parasites. Make wet preparations in saline of large intestinal contents and

Fig. 6.29 Examination of the visceral structures of terrestrial species, in this case a juvenile *Testudo hermanni,* traditionally involves removal of the plastron.

This is done by sawing through the bridges of the shell on each side, and is a simple process using a saw blade attached to a high-speed rotor.

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Table 6.8 (*cont'd*)

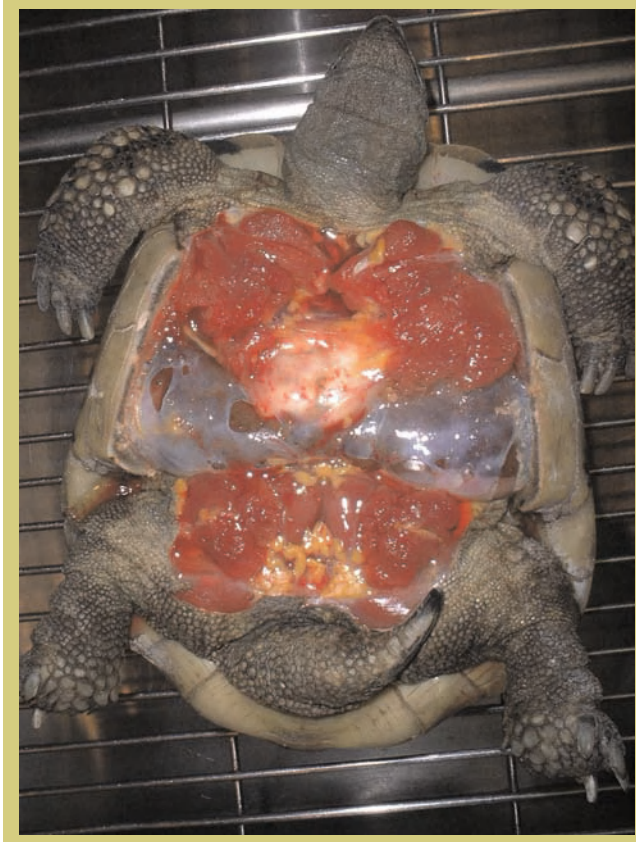

Fig. 6.30 Mature male *Testudo hermanni* with plastron removed.
The pectoral and pelvic muscle masses are clearly seen. The coelomic membrane between them remains and overlies the central liver. Milky white fluid, rich in urates is evident within the pericardium.
This animal died as a result of hyperuricaemia and hyperkalaemia resulting from post-hibernation dehydration.

Fig. 6.31 Subcarapacial abscess in a *Testudo* sp. presented following its sudden death. This animal was almost certainly septicaemic.

Fig. 6.32 Necrotic visceral fibropapillomas in *Chelonia mydas*.

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Table 6.8 (*cont'd*)

Fig. 6.33 The same animal as in Fig. 6.25. Extensive pericardial gout is obvious. The heart is just cranial to the two large liver lobes and just caudal to the pectoral musculature.

Fig. 6.34 This juvenile *Testudo ibera* was presented with chronic anorexia. Ultrasonography suggested a large coelomic mass. Endoscopic biopsy suggested a hepatic adenocarcinoma and the animal was examined following euthanasia.

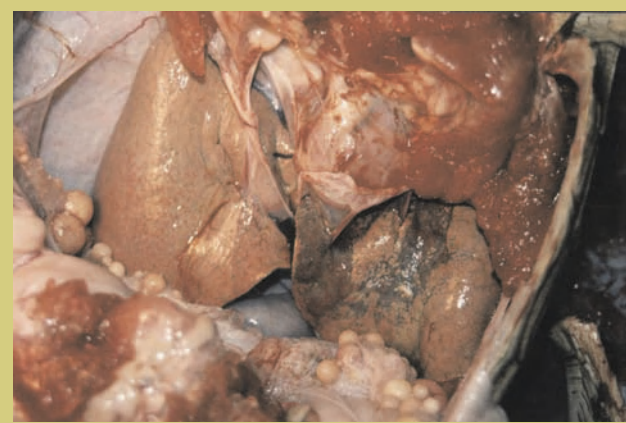

Fig. 6.35 Hepatic lipidosis in a chronically anorexic *Geochelone pardalis*.

examine for helminth eggs and protozoal parasites by microscopy. For identification of gastrointestinal parasites refer to more detailed texts such as Klingenberg (1993) and Klingenberg (1995) or Barnard & Upton (1993).

Respiratory system: Examine the pleuroperitoneum separating the lungs from the other viscera. Open the trachea and follow the short distance into the paired bronchi. The paired lungs located dorsally under the carapace collapse on opening. They are relatively elastic with a honeycomb appearance. Examine them for signs of congestion, haemorrhage or abscess/fibriscessation (Fig. 6.36).

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Table 6.8 (*cont'd*)

Fig. 6.36 Mycotic granuloma in the lung tissue of a chronically anorexic *Geochelone pardalis*.

Fig. 6.37 Kidneys (large and yellow) surround the testes in an animal with several renal gouty tophi.

This animal died of hyperkalaemia as result of renal failure. Renal failure was the result of extensive gouty deposits throughout all renal tubules resulting in their rupture and loss.

Urogenital system: examine the reddish kidneys and the large, sac-like, thin-walled urinary bladder which may be shaped into two or more lobes. Note any urate deposits or calculi (Fig. 6.37). Shell out the kidneys and remove sections for fixation in formalin and freezing. In males the oval testes lie adjacent to the kidneys with the vasa deferentia running next to the ureters to the urodeum. The male copulatory organ can be extruded through the cloaca and examined. In females, the paired ovaries and oviducts will be prominent, especially if mature follicles are present. Examine the shell gland and any eggs present. Inspect the cloaca and note any mucosal inflammation or discharge.

Brain and CNS: remove the skin of the head, find the foramen magnum and cut around the cranium to expose the brain. Shell it out for fixation in formalin.

Musculoskeletal system: open and look at the joints, noting the appearance of the articular surfaces and the presence of any urates. Take samples of joint fluid for bacteriology.

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- *Monocytes*: similar to mammalian counterpart; large cells; nucleus often bean-shaped; lots of pale-blue cytoplasm which may be vacuolated and granulated.
- *Azurophils* (also known as *neutrophils* or *azurophilic neutrophils*): variably present. Medium-sized with an irregular margin; nucleus usually oval. Cytoplasm is variable but often has a dull lavender overall tone and a coarse texture with red–purple granules.
- *Heterophils*: large granulocytes with an eccentric, sometimes lobed, nucleus. Granules are dull red and spindle-shaped. Toxic changes are important to look for and include dark blue granules.
- *Eosinophils*: smaller granulocytes with brighter orange, round granules. May be completely absent.
- *Basophils*: similar to mammalian basophils. Small cells with deep blue granules hiding the nucleus. Much commoner in freshwater turtles.

Performing a total red blood cell (RBC) count

One author (JM) uses the Unopette® 5851 system (BD), in an improved Neubauer counting chamber, for counting RBCs. After sedimentation, five small central squares are counted. When multiplied by 10 000, this gives you the number of RBCs per µl.

Another method would be using Natt and Herrick's solution in conjunction with the Neubauer haemocytometer. 10 µl of blood are mixed with 1.99 ml of Natt and Herrick's solution and incubated for 15–30 minutes. The haemocytometer is filled and after settlement the cells are counted in five small central squares. When multiplied by 10 000 it gives the number of RBCs per µl.

Packed cell volume (PCV)

Use regular micro-haematocrit tubes.

Performing a total white cell (WBC) count

Automated counters are not currently available for this task. We thus rely on somewhat time-consuming and potentially unreliable manual methods. One author (JM) utilises the Unopette® 5877 system and Natt and Herrick's solution.

Heparinised blood (20 µl) is mixed with 1.6 ml Rees and Ecker's stain (available from Leeds Veterinary Laboratories, UK) and incubated for 15 minutes before filling a Neubauer counting chamber. After another 15 minutes the white cells can be counted under × 10 magnification. It can be very difficult to distinguish thrombocytes from lymphocytes. This author (RJW) recommends including thrombocytes in the total count and then performing a correction based on examination of a blood smear (count thrombocytes concurrently during differential white cell count).

Thrombocyte count

There is no reliable method for direct thrombocyte counts. One can perform estimated counts only when the thrombocytes are evenly distributed and not clumped. Multiply the average number of thrombocytes observed in a field by the square of the magnification of the objective used. This gives the estimated number of thrombocytes per µl.

Bacteriology

Avoid contamination of submission material. Anaerobes are important in chelonians. Mixed infections are also common. It is important to preserve samples in such a way as to ensure their survival (in suitable transport medium such as Amie's medium) and specifically to request both aerobic and anaerobic culture from the laboratory.

Opportunistic *Enterobacteriaceae* (*Pseudomonas, Proteus, Klebsiella, Morganella*, etc.) are the common reptile pathogens. They are often resistant to antibiotics. Request sensitivity tests to antibiotics such as fluoroquinolones, ceftazidime, amikacin and ticarcillin.

Mycoplasma isolation and Mycoplasma polymerase chain reaction (PCR) identification

With isolation, false negative results are highly likely unless appropriate sampling techniques, swabs and mycoplasma transport medium are used. Appropriate temperature management and rapid-chilled transportation of samples to a laboratory experienced in the isolation of mycoplasma are essential.

Concurrent bacterial infections may overwhelm mycoplasma colonies. This author (SM) has found that ocular swabs have proved more versatile than nasal/oral swabs in this respect, as bacterial contamination has proved less.

Mycoplasma culture requirements are extensive and culture time is often prolonged. Three or more weeks may be required before colonies are apparent. However, PCR samples may reduce the likelihood of false negative results and the time taken to turn around diagnostic data is greatly reduced.

A sampling technique (used by JM) for mycoplasma PCR on material harvested from the nasal cavities consists in retrograde flushing of the nose from the palate using a small blunt curved probe and normal saline. The tortoise is turned on its carapace and the head tilted downwards while a receptacle (Eppendorf tube) is placed under the nares. The last drops of fluid can be emptied from the nares by massaging the intermandibular space in the direction of the nares while the mouth is kept closed. These samples are then submitted for PCR.

Collecting samples where mycobacterial infection is possible

This is applicable to all cutaneous and subcutaneous nodules and to discrete visceral lesions.

Biopsies (or the excised mass) should be divided into three parts. One should be submitted for histopathology with a request to examine for acid-fast organisms. One should be immersed in transport medium and submitted for bacteriology and the third should be preserved in a sterile container and submitted to an appropriate laboratory for specific mycobacterial culture. The latter may be retained pending histopathology before submission.

All too often the entire sample is sent for histopathology and, on receiving confirmation of acid-fasts, the lesion must be re-biopsied for a sample for culture (empirical treatment is the alternative).

In-house diagnostic testing is possible using an acid-fast staining kit (e.g. TB stain kit ZN®, BD). It is always important to check the whole smear and see if the smear wasn't too thick,

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which could lead to staining artefacts and false positive (failing decolourisation or counterstaining) results.

Cytology

Collecting and preparing samples for cytology is difficult to do well. *Be really gentle!*

- You don't need a vast amount of material but cell preservation is everything.
- Don't rub or smear. The world's greatest reptile cytologist will not save you if the cells are ruptured!
- All slide preparations would be best fixed if not to be stained until later. Methanol (usually included in rapid stains as 'solution 1') is suitable.

Touch preparations

These are suitable for solid masses or internal organs after excision, oral cytology and skin surfaces.

Blot *all* blood from the surface using tissue paper. Just the lightest of touches is required from the surface to be examined onto the slide (or vice versa)—don't rub it! Multiple touches are best—as many as will fit on the middle third of the slide. In living tortoises oral cytology is usually best taken with a cotton bud unless the tongue can be dabbed onto the middle part of a slide. The cotton bud should be *rolled* gently around the mucosa and then *rolled* gently onto the slide. It is vital to achieve rapid drying by making the preparation thin and by using a hair dryer or regular heat fixation.

Fine-needle aspirates

These are suitable for solid lesions of skin, subcutis, lung and coelomic organs.

Use a 19G needle attached to a 5 ml syringe. Stab the lesion, aspirate gently 2–3 times and withdraw. Disconnect syringe and fill with air. Reconnect to needle (in which sample resides) and expel onto centre of a clean slide. Inspect the sample. If a fine spray of droplets is created then leave this part well alone. If you have a dollop of solid matter then lower a second slide flat onto it and gently drag the two in opposite directions horizontally to spread the sample away from the 'spray zone'. Alternatively, using the sample needle, draw out several 'rays' of material.

If the sample is very liquid then it can be treated like a blood smear. In all cases, rapid drying will preserve cell detail better. Thin smears dry faster and the use of a hair dryer is recommended. The prudent clinician will also squirt a sample onto a sterile swab to be kept in medium for bacteriology (aerobic and anaerobic) should this prove necessary.

Coelomic or joint effusions

Very cellular (visibly turbid) exudates can be smeared directly from a droplet using a blood smear technique. It is vital to achieve rapid drying by making the smear thin and by using a hair dryer. Less cellular samples can be centrifuged for a few minutes to concentrate a pellet which is then aspirated into a pipette, deposited on a slide and gently smeared using a cover slip or slide lying flat on top of the sample.

Alternatively, a sample can be preserved according to external laboratory requirements (some prefer formalinised, some EDTA) and dispatched. Either way a sample should also be preserved for bacteriology (including part of it on a swab in transport medium) in case this should prove necessary.

Bronchial washes

Instil 0.5–2 ml of sterile saline via a catheter (inserted through an endotracheal tube to prevent contamination of the sample from the upper respiratory tract) and then aspirate back as much fluid as possible with a 10 ml syringe. The most useful part of the sample will be in the catheter. Disconnect the syringe and fill with air. Reconnect and expel sample into a sterile container.

Where a sample is visibly turbid, pieces of solid matter should be aspirated using a sterile pipette, deposited on a clean slide and gently smeared using a cover slip or slide lying flat on top of the sample. It is vital to achieve rapid drying by making the smear thin and by using a hair dryer.

Less cellular samples will need centrifugation to concentrate a pellet. If the sample is of very low cellularity then it is probably better to use the services of a laboratory with a centrifuge capable of spinning the sample down directly onto a slide. In all cases the remainder of the sample should be preserved (part of it on a swab in transport medium) for bacteriology and/or virology.

Cerebrospinal fluid (CSF)

These samples are usually of very low cellularity and are best preserved for transport to a laboratory with a centrifuge capable of spinning the sample down directly onto a slide. In all cases part of the sample should be preserved (part of it on a swab in transport medium) for bacteriology and/or virology.

Staining cytological preparations

This author (RJW) routinely uses a rapid Romanowsky stain (Rapi-Diff® II, Diachem International), which is very easy to use and adequate for the vast majority of situations. It is also important to have available an acid-fast stain for screening samples in which mycobacteria may be present. The Ziehl-Neelsen staining protocol is also suited to a general practice laboratory. Make sure the smear is thin in order to avoid staining artefacts with acid-fast stain and getting false negative results. On occasion it may be desirable to characterise micro-organisms present using Gram stain (e.g. 3-Step Gram Stain Kit-S®, BD).

Ziehl-Neelsen method: heat fix the slide (specimen side up) by passing through a flame. Flood with carbol fuchsin. Heat until it steams, then leave for five minutes. Rinse with water. Decolourise with acid-alcohol for 1–2 minutes until red colour has gone. Rinse. Flood with methylene blue for 30 seconds. Rinse and dry. Acid fasts appear red, others are blue. It is always important to check the whole smear and see if the smear wasn't too thick, which could lead to staining artefacts and false positive (failing decolourising or counterstaining) results.

Faecal samples

Wear gloves and treat samples as being infectious to you. Remember that flagellate motility ceases within a day.

Wet smear

Wet smears give a rapid indication of which organisms are present.

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Mix a small amount of sample with a few drops of saline on a slide. Apply a cover slip and examine under × 10 magnification. The addition of a drop of Lugol's iodine will stain amoebal cysts and other organisms. The use of phase contrast microscopy will facilitate the visualisation of protozoans.

Sedimentation

Sedimentation concentrates protozoans and trematodes.

Flotation

Flotation concentrates parasites, although trematode and cestode eggs may not float. Sucrose or zinc sulphate are most often used. Zinc sulphate is better, but crystallises after 20 minutes.

Dried smears

Dried smears are of limited use.

An acid-fast stain may be used for *Cryptosporidia*. Using a modified acid-fast stain with brilliant green as counterstain, *Cryptosporidia* appear dark red on a green background. Rapid stain for yeast overgrowth.

Urine samples

Chelonians often urinate when stressed by handling. Have a receptacle ready! Venepuncture is especially likely to induce voiding. The bladder wall is very thin and, although cystocentesis can be done easily enough through the prefemoral fossa, there is significant risk of subsequent urine leakage. The bladder is not easy to catheterise in a conscious patient although it can easily be accessed using a rigid endoscope in the sedated or anaesthetised patient.

Specific gravity should be measured using a refractometer; pH and the presence of ketones should also be determined using dip sticks. Other parameters are less useful.

Urates (gout)

When a sample containing urate is mixed with nitric acid and then with ammonia a characteristic purple colour is obtained. This test, used by most laboratories to identify urate uroliths in mammalian patients, can be used to identify gout in reptiles. This can be useful since aqueous formalin solutions dissolve the crystals in histopathological samples. However, a significant sample size is required (fine-needle aspirates have proved insufficient) and it should be borne in mind that dystrophic urate deposition can occur in inflammatory lesions such as abscesses/fibriscesses. Cytologically, urate crystals are often amorphous and occasionally rounded, with an 'X' pattern. They must be distinguished carefully from the ubiquitous glove powder starch grains.

Electron microscopy (EM)

Material for examination by electron microscopy can be obtained by direct collection of excreted body fluids such as faeces and urine or by washes to release such material (e.g. respiratory or cloacal washes). Biopsy and necropsy samples are well suited to electron microscopy and can be obtained at post mortem, endoscopic examination or surgically. Fluid from vesicles can be harvested using aspiration through a sterile syringe and needle.

During the course of a viral infection, electron microscopy may only identify the presence of viral particles if sufficient numbers of viral particles are present and sample material is harvested from specific locations at specific times. Serial sampling during the course of the disease increases the likelihood of obtaining a positive sample.

After collection, samples should be chilled or frozen during storage and transport. Fixatives are not usually required, although sample material should not be allowed to desiccate. Care should be taken to avoid unnecessary contamination of samples by contact with human or other tissues.

Virus isolation

Virus isolation from may allow infections to be diagnosed in situations where virus particles are too scarce to be revealed by EM, although where available PCR is likely to be most sensitive. If cytology or histopathology gives results consistent with a viral infection, then submission of swabs for virus isolation may be justified, even in the absence of positive EM evidence.

Heavy bacterial contamination of swabs, such as is likely with faecal and oral swabs, may denature any virus present, resulting in false negative results, as may inappropriately high temperatures during storage and transportation.

Samples for virus isolation can be harvested from excreted material, body fluid, dry swab specimens of lesions or lumenal surfaces or tissue biopsies (Jacobson 1999b). An aseptic technique is advisable.

Once harvested, material should be isolated from potential contamination in sealed, sterile containers or bags, stored at appropriate low temperatures (~5°C) and transported to the virology laboratory chilled. Further advice should be sought from the virology laboratory.

Freezing to temperatures at or below minus 40°C suits long-term sample storage, although infectious units will be significantly decreased in comparison to storing chilled (Marschang: personal communication 2001).

Serology

As methods of serological diagnosis are likely to advance rapidly over the next few years, it would be wise to contact laboratories offering serological testing for chelonian herpesvirus regarding current submission requirements. At the time of writing, this author uses heparinised jugular blood samples, which are then centrifuged, and the plasma subsequently removed by pipette. Following decanting the plasma is frozen and stored or transported chilled to the laboratory for testing (e.g. virus neutralisation).

Molecular tests (PCR)

Molecular tests such as PCR can be applied to virtually any excreted material, body fluid, dry swab specimens of lesions or lumenal surfaces and tissue biopsies. Materials should be collected as described. PCR is less affected than virus isolation by inappropriate temperatures during transportation and storage.

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Table 6.9 Additional techniques, suited to marine turtles (Whitaker & Krum 1999).

Coelomocentesis	Fluid resulting from hepatic, renal or cardiovascular disease can be sampled by inserting a needle of suitable length into the prefemoral fossa. This technique will also reveal and allow drainage of free air in the coelomic cavity.
Harvest of cerebrospinal fluid (CSF)	Similar to mammalian *cisterna magna* tap.
Blood collection	Some advice is given under venepuncture and phlebotomy sites below. Some authors now advise against cardiocentesis as too dangerous. *Dorsal cervical sinus:* • most commonly used site—provides suitable quantities of blood with minimal difficulty; • head positioning involves either the use of a table or placing the turtle on thick foam or a table edge and then tipping the head downwards and forwards to expose the back of the head; • paired vessels lie on the dorsal nuchal aspect, either side of the midline, becoming more lateral with emaciation; • vessel location can be facilitated by ultrasound in suitably-sized individuals; • sampling is possible anywhere between the caudal aspect of the skull and the cranial carapacial inlet. *Jugular vein*: similar to other chelonians and as described below. *Metatarsal vein*: located centrally in the dorsal aspect of the metacarpus.

Immunohistochemistry

Sample collection as for cytology. Excreted material, body fluid, dry swab specimens of lesions or lumenal surfaces or tissue biopsies are all appropriate.

Marine turtles

Table 6.9 details some additional techniques.

VENEPUNCTURE

Examination of blood should be part of a routine work-up of any chelonian case. Clinicians are advised to attempt jugular venepuncture whenever possible. Should this prove impractical, the subcarapacial (subvertebral) and dorsal tail sites may then be investigated. It should be borne in mind that a jugular sample is least likely to be lymph diluted. Venepuncture sites are illustrated in Figs 6.38–6.61. Skin disinfection should be undertaken. These sites are also available for intravenous (IV) injection in situations such as critical fluid therapy.

Suggested collection protocol

Collect 1–1.5 ml/kg bodyweight of blood. This is usually sufficient to run an extensive panel of chemistries and haematology. A maximum of 3 ml/kg is advised, although such a volume is seldom necessary. Some clinicians (JM) use EDTA in addition to lithium-heparin to preserve samples for haematology, provided they are processed soon after collection, as white blood cells tend to aggregate in lithium-heparin. RBC count is performed from lithium-heparin samples only.

Take two lithium-heparin samples: the first for haematology; the second should be centrifuged, separated and frozen immediately after collection for biochemistry (alternatively use a spun lithium-heparin gel tube). Prepare several air-dried smears.

Phlebotomy and venous access sites

Venous access sites described in chelonians include:

- internal and external jugular veins;
- axillary plexus;
- occipital plexus;
- subcarapacial (subvertebral) sinus;
- dorsal venous sinus (tail vein, subarachnoid sinus);
- heart;
- ulnar plexus;
- radiohumeral plexus;
- distal caudal femoral plexus;
- femoral vein;
- a short-clipped nail (this is not recommended–it causes distress and the sample is non-diagnostic);
- orbital sinus.

Many of these sites are unsuitable for harvesting an ethical diagnostic sample because of resulting lymph contamination, potential trauma to associated tissues and unnecessary pain and distress at sampling. Others can be used in some species or sizes, but not others.

Jugular veins

A jugular vein is the sample site of choice in most chelonians weighing less than 4 kg. However, it is often unsuitable for phlebotomy of large chelonians (e.g. >15 kg), or aggressive species such as snapping turtles, without adequate prior chemical restraint, which may in turn affect the reliability of results.

In some species dorsal and ventral veins appear to be present (Thompson 1932). Either will provide a diagnostic sample if cleanly entered. Anatomical texts appear to describe an internal and external jugular vein either side of a large cervical lymphatic vessel: the superficial jugular trunk (Ottaviani & Tazzi 1977) (Figs 6.38–6.41).

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Fig. 6.38 Anatomical site of the jugular vein in a Greek Tortoise (*T. hermanni*).

Note its dorsal location in extension of the dorsal rim of the tympanic membrane. The vein appears directly caudal to the cartilaginous prominence of the hyoid apparatus.

The dorsal jugular vein (1) is associated with the carotid artery (2), the jugular lymphatic trunk (3) and the ventral jugular (4).
[Courtesy of Jean Meyer]

Fig. 6.39 The jugular vein can be raised and seen in many chelonians.

The right jugular is generally easier to sample than the left for right-handed operators. Few authors advise raising the vein during the sampling procedure.

Here the vacuum in the syringe is exaggerated for the purpose of the picture. When taking a diagnostic sample care should be taken to prevent damage to cells by applying excessive suction.

Fig. 6.40 Jugular and carotid sampling are easiest with the animal restrained by an assistant and tipped into semi-lateral recumbency. The assistant will hold the animal in a steadier manner with their elbows anchored on a work surface.

Here the vacuum in the syringe is exaggerated for the purpose of the picture. When taking a diagnostic sample care should be taken to prevent damage to cells by applying excessive suction.

Fig. 6.41 After sampling from any vessel in the neck it is advisable to apply digital pressure to the site to reduce haematoma formation.

Haematoma formation is most likely if the carotid artery, as opposed to the jugular vein, is entered. A carotid sample appears to be of reliable diagnostic quality.

To sample from a jugular vein:

(1) Allow an assistant to stand opposite, across a table with their elbows resting on the table-top. The assistant presents the chelonian head first towards the clinician. It may be easiest if the assistant holds both of the forelegs back.

(2) The head is drawn out into extension by the clinician, and a finger and thumb used behind the occiput to restrain the neck and head.

(3) The chelonian can then be tipped into lateral recumbency. This exposes the lateral neck. This right-handed author (SM) usually uses the right hand side of the animal's neck.

(4) The animal will be kept stable by the restraining assistant resting on a table-top or surface whilst supporting the animal.

(5) In some species dorsal and ventral veins appear to be present (Thompson 1932). Either will provide a diagnostic sample if cleanly entered. Anatomical texts appear to describe an internal and external jugular vein either side of a large cervical lymphatic vessel: the superficial jugular trunk (Ottaviani & Tazzi 1977).

(6) The jugular vein is often visible as it extends caudally from the angle of the mandible, occasionally paired. The jugular vein is relatively easily sampled by inserting a needle attached to a syringe very superficially in a caudal-pointing direction using a resting point on the extended head to keep everything steady. The dorsal vein runs approximately in a line drawn from the dorsal edge of the tympanic membrane parallel to the dorsal plane of the neck in the direction of the carapacial inlet. When tilting the head

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slightly away from the phlebotomy site with the head in extension, a cartilaginous prominence can be felt a few millimetres laterally, behind the head. The jugular vein appears directly caudally to this prominence.

(7) The sample site should be thoroughly disinfected.

(8) Avoid excessive compression/raising of the vessel when taking the sample, as this may lead to the formation of a large haematoma after removal of the needle.

(9) Occasionally, the carotid artery is entered if the needle is inserted into the proximal neck towards the midline. Here the syringe will fill rapidly with noticeably bright red blood. According to Jacobson (1992), the carotid artery is deeper, ventral and parallel to the jugular vein. It is acceptable to sample the carotid artery, but techniques to ensure adequate haemostasis, as described below, must be employed after the needle is withdrawn.

(10) Following sampling from any vessel in the neck, a pressure pad of cotton wool (or similar) should be applied for several minutes in order to reduce post-sampling haematoma formation. This is especially the case if the clinician suspects that an arterial supply has been entered.

(11) Post-sampling haematomas are relatively unimportant but may cause keepers distress. Most usually resolve within two weeks.

Jugular catheterisation

Jugular catheterisation has been described by Jacobson *et al.* (1992) and Lloyd & Morris (1999), who advocate the use of a 23G–25G butterfly needle. Alternatively, a catheter may be used and flow-line® tubing can be secured to the carapace allowing attachment of a syringe driver (Figs 6.42–6.44).

Dorsal venous sinus (dorsal coccygeal vein)

In many species, the dorsal venous sinus is found in the dorsal midline of the tail and may run virtually from the tip of the tail to the carapace (Figs 6.45–6.46).

Ippen & Zwart (1995) found that chelonian tail anatomy varied between both species and individuals. These authors also concluded that this sample is unreliable because of unpredictable lymphodilution and suggested that venepuncture may give rise to potential damage of the central nervous system if the subarachnoid space is entered and CSF is sampled without adequate disinfection.

Fig. 6.42 Cut-down and exposure of the right jugular vein in a severely dehydrated and collapsed *Testudo hermanni.*

Fig. 6.43 Insertion of 23G catheter into jugular vein.

Fig. 6.44 Temporary placement of stay sutures before attachment of catheter to a syringe driver.

The vessels of the tail may be at least partially drained by the renal portal vein as described earlier. Therefore the renal tubules may be exposed to material carried in the blood from the tail. The implication is that renal infections may result from poor aseptic technique when taking blood samples from the tail.

Often this is the only available site in some individuals, so, despite its disadvantages, is utilised out of necessity:

(1) Allow an assistant to stand opposite, across a table with their elbows resting on the table-top.

(2) This assistant presents the caudal aspect of the upright chelonian towards the clinician.

(3) The sample site should be thoroughly disinfected.

(4) With a stabilising hand holding the disinfected tail in the midline, the clinician inserts a 21G–25G needle (attached to a 2 or 5 ml syringe) slowly, at an angle of around 45°, whilst applying gentle suction.

(5) When the sinus is located, the syringe should fill easily with blood.

(6) If clear fluid is sampled, suction should be abandoned and another attempt made at a different site.

(7) If the sinus is unyielding or not located in the distal tail, then one can work more proximally.

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Fig. 6.45 Injection of propofol (Rapinovet®, Schering-Plough; Diprivan® Zeneca) into the dorsal venous sinus of a male marginated tortoise (*Testudo marginata*).

This site requires considerable disinfection and, whilst often convenient, may not be as suitable as the jugular vein.

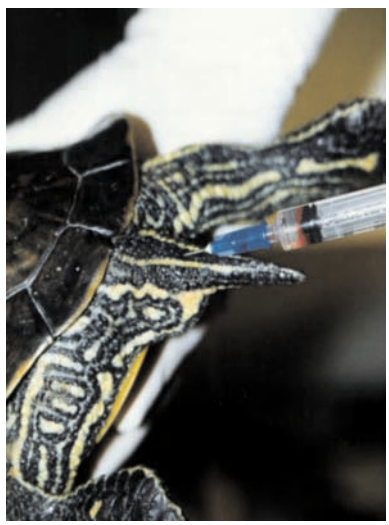

Fig. 6.46 Lymphodilution makes results of blood analysis of samples taken from the dorsal coccygeal vein unreliable.

Similarly, lymphodilution is likely in samples from the subcarapacial or subvertebral vein and lateral occipital vein. This author suggests that jugular or carotid samples are taken whenever possible.

(8) A tortoise that refuses to expose its tail can often be sampled by inserting the needle blind, in the midline.

(9) The vessel is occasionally more superficial than expected.

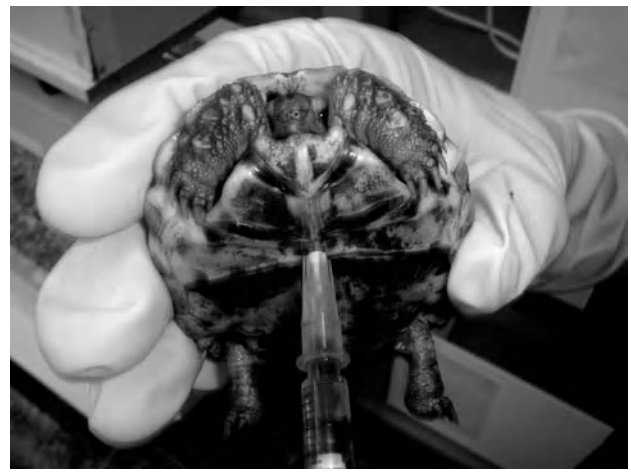

Fig. 6.47 Cardiac sampling in a juvenile tortoise with an inadequately calcified plastron.

This appears to be a particularly invasive technique, prone to complications such as myocardial damage or septicaemia. However, in the limited number of animals this author has sampled in this manner, no such complications arose. It has been favoured by some authors, but may be less suitable than a subcarapacial sample in animals where jugular or carotid sampling appear unrewarding.

Fig. 6.48 Cardiac venepuncture and injection of barbiturate during euthanasia procedure.

This technique utilises a long needle inserted lateral to the head through the cranial carapacial soft tissue window.

Cardiocentesis

There are varying comments regarding patient safety and ethical issues regarding analgesia when using this site. Jacobson (1992) advises against cardiocentesis on the grounds that there is a high risk of inducing a bacterial pericarditis. However, Boyer (1998) suggests this is the most practical site for chelonians weighing less than 50 g (Figs 6.47–6.48).

This author (SM) would tend to use the subcarapacial or subvertebral sinus in such small animals but has not experienced obvious complications when sampling blood from the heart of small juvenile animals with relatively soft plastrons. Small, soft-shelled hatchlings or neonates sampled have not demonstrated distress or pain to the author when the shell was pierced, although large animals have been observed to thrash about if the plastron is merely touched with a needle. Analgesia is therefore an important issue here. This author would advise that any animal demonstrating a pain response when a needle is touched against the plastron

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should not be sampled with this technique or should be anaesthetised before needle or drill insertion. It cannot be guaranteed that blood samples taken from anaesthetised animals will give valid diagnostic parameters.

To access the heart:

(1) Anaesthesia must be considered, as outlined above.
(2) The heart is dorsal or just anteriodorsal to the junction of the humeral abdominal scute intersection of the plastron. Boyer (1998) points out that allowance should be made for anterior movement of the heart when the head is extended.
(3) After suitable sterilisation a hole is pierced in the plastron at this location, using a 25G needle with the patient in dorsal recumbency. This avoids premature puncture of the heart, which will fall away from the plastron puncture site.
(4) By placing the patient back into ventral recumbency, the heart will contact the plastron in the region of the puncture site and can be sampled by insertion of a smaller-gauge needle upwards through the guide hole.
(5) The plastron can then be sealed using tissue adhesive or a similar material.

Rooney *et al.* (1999) also describe semi-permanent cardiac venepuncture:

(1) An 8.46 mm hole was drilled in the plastron of anaesthetised desert tortoises (*Gopherus agassizii*) in the midline, at the junction of the pectoral and humeral scutes.
(2) A self-sealing 3 mm rubber bung (Vacutainer, BD), was fixed in place within this hole with five-minute epoxy resin.
(3) Animals were sampled in ventral recumbency, through the bung, without obvious adverse effect.

This technique would appear to suit animals repeatedly sampled over time in research institutions.

Lloyd & Morris (1999) describe a third technique where the needle is inserted through the thoracic inlet. This author often uses this site when performing euthanasia, but has never sampled animals from this site as it would appear unnecessarily traumatic:

(1) In a 2 kg tortoise sedated with a concentrated intramuscular ketamine solution (100–200 mg/kg), a 19G, 1.5 inch needle, attached to a syringe containing a concentrated barbiturate euthanasia solution, is inserted centrally in the clavicular fossa.
(2) This author (SM) tends to use the animal's left hand side.
(3) The needle is advanced and directed towards the presumed position of the heart, which is ventral to the saggital midline, about a third of the way down the animal's plastron, whilst applying gentle suction.
(4) Upon entry of the heart blood is readily recovered in the syringe and the euthanasia injection (usually concentrated barbiturate solution) can be given.
(5) Animals are then pithed, as without this it is possible for any cardioplegic drugs administered to be metabolised, resulting in recovery at some later point.

Euthanasia is covered in greater detail in Chapter 14.

Dorsal cervical sinus

This site is popular with those working with marine chelonians. The technique is well described (Bentley & Dunbar-Cooper 1980; Owens & Rutz 1980: Lutz & Dunbar-Cooper 1987; Bolten & Bjorndal 1992) (Figs 6.49–6.52).

Fig. 6.49 Sampling from the lateral occipital sinus of a marine turtle. (Courtesy of Prof E.R. Jacobson, University of Florida)

Fig. 6.50 Vacutainers® are suited to sampling from the lateral occipital sinus of marine chelonians.
(Courtesy of Prof E.R. Jacobson, University of Florida)

Fig. 6.51 A neck-restraining crush box can assist when sampling the occipital sinus of marine turtles.
(Courtesy of Prof E.R. Jacobson, University of Florida)

(1) A needle is inserted perpendicular to the dorsal neck and marginally away from the midline (0.5–1 cm).
(2) The paired sinuses are found 0.5–3 cm deep depending upon the turtle's size.
(3) The head is best maintained lower than the body and the use of a restraining cradle decreases the need for operator handling.
(4) Vacutainer tubes or heparinised syringes are suitable for use with larger turtles.

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Fig. 6.52 Care should be taken when handling the heads of unsedated or unrestrained marine chelonians as they are capable of inflicting serious injury with their beaks.
(Courtesy of Prof E.R. Jacobson, University of Florida)

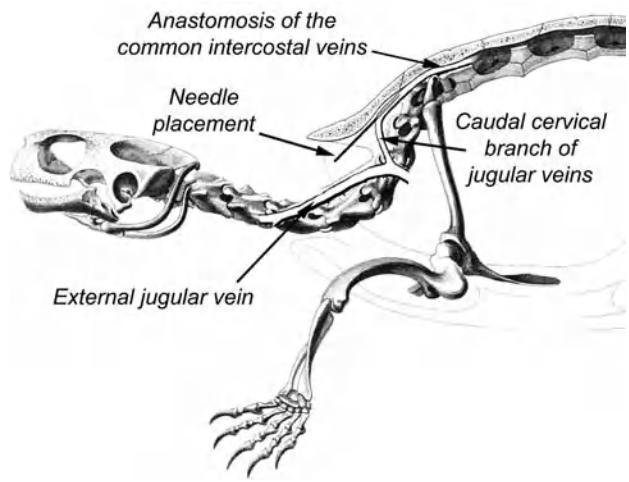

Fig. 6.53 Diagram illustrating the relationship between the carapace, cervical spine and venous circulation of a chelonian, including the placement of the needle for venepuncture (adapted from Bojanus 1819).
(Courtesy of the Journal of Herpetological Medicine and Surgery and Stephen J. Hernandez-Divers, University of Georgia.)

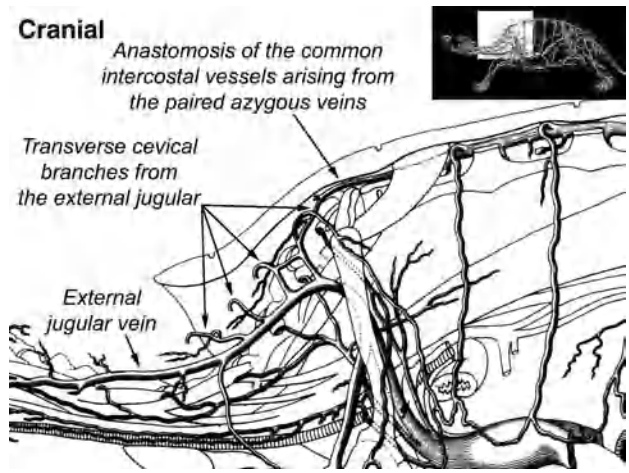

Fig. 6.54 Lateral view of the venous circulation of the cranial carapace of a chelonian, depicting the relationship between the caudal transverse cervical branches of the external jugular veins and the cranial anastomosis of the common intercostal vessels that arise from the azygous veins (adapted from Bojanus 1819).
(Courtesy of the Journal of Herpetological Medicine and Surgery and Stephen J. Hernandez-Divers, University of Georgia)

Gottdenker & Jacobson (1995) and Lloyd & Morris (1999) describe the use of this site in terrestrial species. Due to the ventroflexion of the head, the dorsal space between atlas and occiput is widely open, which makes the spinal cord prone to potentially lethal damage. This technique should be used, therefore, with great caution when the clinician is inexperienced.

(1) The animal is restrained in sternal recumbency with its head over the edge of a table.
(2) The head is held extended from the body and tipped in slight ventroflexion.
(3) The needle is inserted behind the occiput, in a caudal direction, at an angle of 30°.

Subcarapacial (subvertebral) venous sinus

This site is very useful, especially in situations where other sites prove too difficult.

Hernandez-Divers *et al.* (2001) and Hernandez-Divers *et al.* (2002) recently identified anatomical structures associated with subcarapacial vessels. Angiographic studies using radio-opaque iohexol, static radiography and video fluoroscopy were undertaken in several chelonian species. Latex-injected dissections were also performed to confirm the vascular anatomy. The authors demonstrated that a clinically-useful venepuncture site is present in the anterior dorsal midline at the junction of the common intercostal veins and the caudal cervical branch of the external jugular veins, just cranial to the last mobile cervical vertebra.

The target area under the carapace, caudal to the nuchal scute, at the level of the eighth cervical vertebra (Hernandez-Divers *et al.* 2002) contains a variety of vessels including the nuchal lymphatic sinus, the internal jugular vein, an anterior pulmonic vein, vertebral veins, the subclavian vein and of course their related arterial and lymphatic vessels (Thompson 1932; Ottaviani & Tazzi 1977; Hernandez-Divers *et al.* 2002) (Figs 6.53–6.55).

No studies yet clarify how blood taken from this area is likely to be affected by lymphodilution or pooling associated with the vascular dive reflex described earlier, and a comparison of blood taken simultaneously from this site and the jugular vein is required before a sample from this area can be guaranteed to be of full diagnostic value. Waters (personal communication 2002) describes work showing statistical variance between blood taken from the jugular vein and the subvertebral venous sinus of chelonians and it is his opinion that the subvertebral site is non-diagnostic (Figs 6.56–6.57).

Sampling from the subcarapacial sinus can be done with the neck extended or flexed:

(1) This author tends to use a 23G, 1–2-inch needle, or the stylet from an intravenous catheter. It is sometimes helpful to bend the needle prior to insertion.
(2) Insert a needle dorsal to the neck angled upwards at around 60°, whilst applying gentle negative pressure.
(3) If a vertebra, or the underside of the carapace, is encountered the needle is withdrawn slightly and redirected cranially or caudally, whilst still applying gentle negative pressure.

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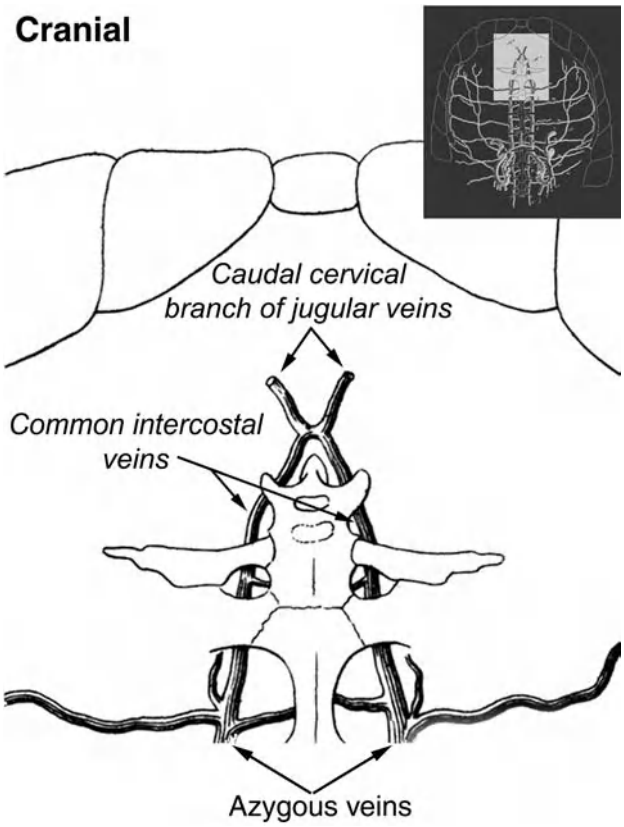

Fig. 6.55 Dorsal view of the venous circulation of the cranioventral dorsal carapace of a chelonian depicting the relationship between the caudal transverse cervical branch of the external jugular veins and the cranial anastomosis of the common intercostal vessels that arise from the azygous veins (adapted from Bojanus 1819).
(Courtesy of the Journal of Herpetological Medicine and Surgery and Stephen J. Hernandez-Divers, University of Georgia)

Fig. 6.56 Intravenous injection of propofol (Rapinovet®, Schering-Plough; Diprivan®; Zeneca) into the subvertebral vein of a juvenile spur-thighed tortoise (*Testudo graeca*).

(4) If lymph is sampled, the procedure should be abandoned in favour of a fresh attempt using new materials. Lymphatic vessels lie just cranial to the target area for sampling.

Other sites

There are other sites, but they are generally of less importance.

Fig. 6.57 The subvertebral site can be accessed with the head and neck in either flexion or extension.

Fig. 6.58 Site of the veins on the caudal aspect of the forelimb of Hermann's tortoise (*Testudo hermanni*).
The cephalic vein is a sampling site of lesser importance, a jugular vein being the preferred sampling site. This vein may be more yielding in larger animals.
(Courtesy of Jean Meyer)

Ulnar (radiohumeral) venous plexus

This site is described by Rosskopf (1982). Boyer (1998) describes the site as useful in chelonians weighing over 1 kg. Blood values of samples from this site in normal *Testudo hermanni* are given by Göbel & Spörle (1992). Lymph contamination is likely at this site (Jacobson 1992).

To access this site, extend the front leg and palpate the prominent tendon on the caudal aspect of the radiohumeral joint. With the leg extended, the vessel lies between the tendon and the joint, cranial to the tendon and behind the superficially visible tendon. Insert a needle just caudal or ventral to this tendon, perpendicular to the skin and angled towards the radiohumeral joint (Figs 6.58–6.59).

Femoral plexus

Boyer (1998) describes the site as similar to the ulnar plexus, but in the hind limb.

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Fig. 6.59 Technique of cephalic venepuncture in a venepuncture in a Hermann's tortoise (*Testudo hermanni*).
(Courtesy of Jean Meyer)

Femoral vein

Injection into the femoral vein is described by Holz (1994) and sampling of blood from this site by Lloyd & Morris (1999) (Figs 6.60–6.61). Extension of the pelvic limb, with the animal in dorsal recumbency, exposes the femoral triangle. The vein, which these authors suggest is difficult to find in most species, lies almost directly over the bone, near the plastron.

Nail clip

Avoid this site at all costs as sample quality is completely unreliable and sampling may cause distress to the patient (like cardiocentesis). Used by early authors such as (Lawrence & Hawkey 1986).

Orbital sinus

The orbital sinus can be used for collection of small blood samples using capillary tubes (Nagy & Medica 1986). A capillary tube is placed into the lateral/temporal canthus of the eye and twisted. Jacobson (1992) suggests caution as trauma may be caused to periocular and corneal tissues and lymph dilution is likely.

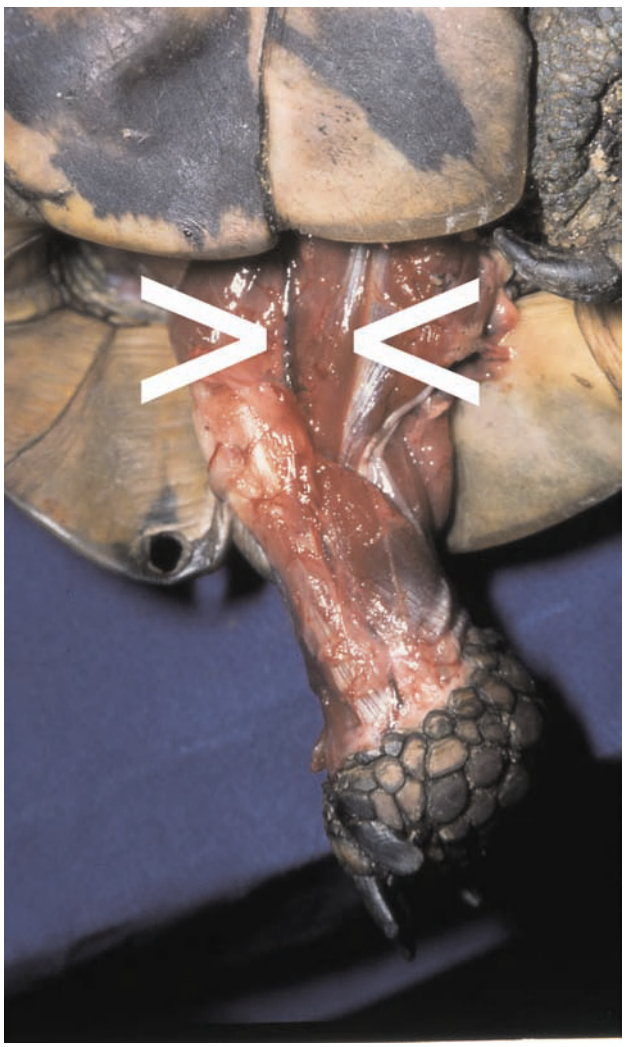

Fig. 6.60 Anatomical relationships of structures about the femoral vein of Hermann's tortoise (*Testudo hermanni*).

The femoral vein is a venepuncture site of lesser importance, a jugular vein being the preferred sampling site. This vein may be more yielding in larger animals.
(Courtesy of Jean Meyer)

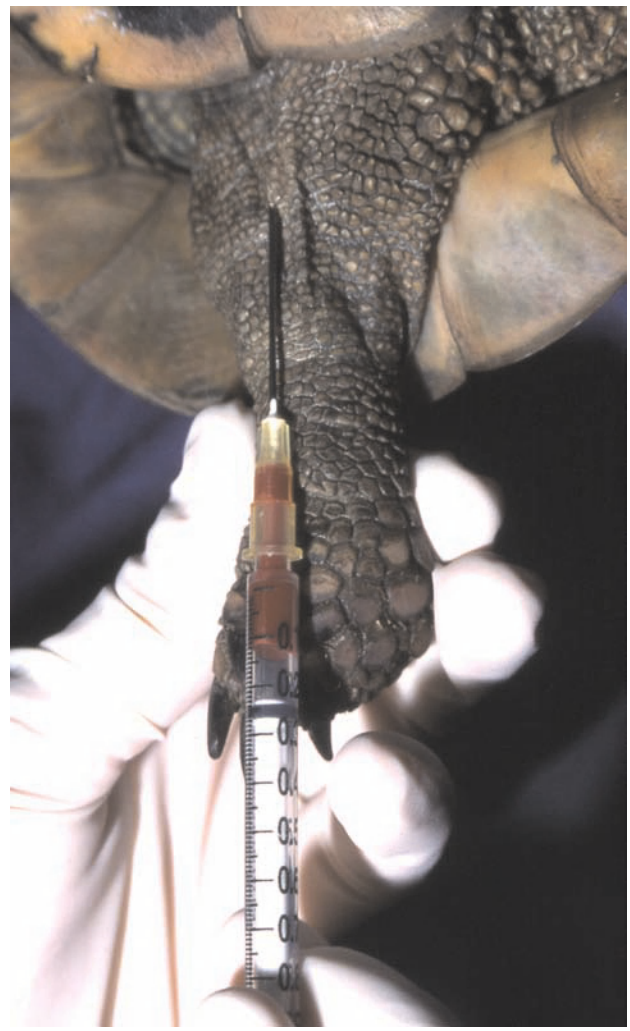

Fig. 6.61 Technique of femoral venepuncture in a Hermann's tortoise (*Testudo hermanni*).

Only small, unpredictably lymphodiluted samples can be obtained from this site.
(Courtesy of Jean Meyer)

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In addition to the sources referred to above, further comments on sampling sites in chelonians have been made by: Richter *et al.* (1977a); Richter *et al.* (1977b); Samour (1984); Jacobson (1993a); Mautino & Page (1993); Jenkins (1996); Kolle & Hoffman (1996); Murray (2000).

For examples of practice biochemistry analysers, see Figs 6.62–6.63.

Fig. 6.62 The Synermed IR202®, Synermed Europe Ltd, 67 Victoria Road, Burgess Hill, Sussex.

This wet chemistry blood biochemistry analyser is used by the author (SM) and appears to produce reliable assessment of all commonly required chelonian blood biochemistry parameters including GLDH and uric acid.

The Synermed IR202® complements a wet chemistry machine such as the Easylyte Calcium® (Fig 6.63).

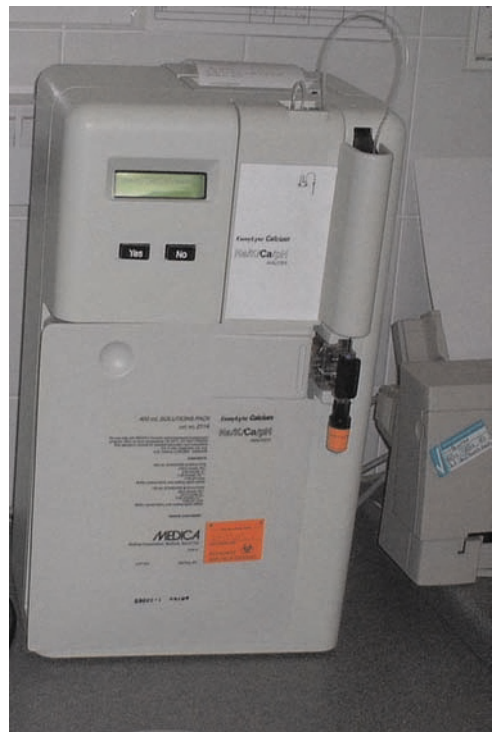

Fig. 6.63 The Easylyte Calcium (Na/K/Ca/pH)®, Medica, Medica Corporation, Bedford, MA.

This machine used by the author (SM) produces rapid, reliable in-house assessment of pH, sodium, potassium and ionised calcium.

The Easylyte Calcium® complements a wet chemistry machine such as the Synermed IR202®.

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CLINICAL PATHOLOGY

ROGER WILKINSON

LABORATORY INVESTIGATION

BLOOD SAMPLING

Heparinised samples are preferred for haematology because EDTA can cause haemolysis in chelonian blood samples (Muro *et al.* 1998b). However, because heparin can cause clumping of leucocytes and thrombocytes, it is wise as well to prepare fresh, thin and rapidly air-dried smears for later staining. EDTA-preserved samples can be made for leucocyte counts if processed quickly. Raskin (2000) recommends that cover-slip smears are less likely to cause cell lysis than the conventional method using a second slide. Citrated blood samples may also be suitable for haematology (Raskin 2000).

Bolten *et al.* (1992) and Jacobson (1992) reported that clotting in serum samples was unpredictable and that plasma is to be preferred to serum for biochemistry. The sample should be separated after collection to avoid haemolysis. In mammals, failure to separate biochemistry samples may, in particular, lead to real elevations in LDH, AST, phosphate and potassium and apparent decreases in calcium and alkaline phosphatase, depending on the method of estimation (Bush 1991). Mader & Rosenthal (1998) found that failure to separate plasma from cells caused elevations in phosphate, potassium and CPK but falls in glucose and cholesterol.

The following, at least, should be collected from each patient:

- one lithium heparinised blood sample;
- one lithium heparinised sample which has been centrifuged, separated and frozen immediately after collection (a lithium heparin gel tube may be used for this sample);
- several air-dried smears.

Whether or not blood smears should be fixed prior to transport should be discussed with the laboratory that will be processing them. In addition, it is important to enquire about the normal values used by the analysing laboratory.

Tables 7.1 and 7.2 below give blood parameters for a tortoise 'profile' and data to be collected for pathology purposes.

Table 7.1 A suggested tortoise profile.

Haematology, including differential white cell count and examination for haemoparasites		
Total protein	Potassium	Uric acid
Albumin	Alkaline phosphatase	Urea
Calcium (total and ionised)	LDH	Beta-hydroxybutyrate
	GLDH	
Phosphate	AST	Biliverdin (if test becomes available)
Sodium	CK	

Table 7.2 Suggested clinical pathology data-collection protocol.

Species	Captive or wild caught
Age	Details of diet and supplements
Sex	Last meal/duration of anorexia
Reproductive status	Weight and length
Season	Previous treatment
Winter hibernated?	Hydration status
Sample site	Stressors
Anticoagulant used	Clinical signs
Time to separation	Presence of parasites

As discussed above, all samples should be accompanied by full details of the circumstances under which they were obtained. Not only will this greatly facilitate interpretation of the sample, but in the longer term will also add to our evolving understanding of chelonian medicine. Most of the following parameters were recommended by Anderson & Wack (1996) for workers carrying out studies of blood parameters in reptiles. Work load permitting, we would urge clinicians to use it for all cases.

Sample volume

Thorson (1968a & 1968b) found that total blood volume accounted for 4.9%–7.2% of body weight in the terrestrial desert tortoise *Gopherus polyphemus*, 3.8%–5.6% in the freshwater turtle *Chelydra serpentina* and 5.2%–7.9% in five species of marine chelonians. Since, in most species, up to 10% of blood volume may be taken relatively safely, approximately 3 ml/kg blood would seem a reasonable maximum sample size for most chelonians. 1–2 ml/kg will usually suffice and will allow most parameters to be assessed.

Mader & Klaasen (1998) investigated the practice of sample dilution and recommended that when this is necessary (because of small sample volume) the dilution, in de-ionised water, should be no more than 1:3 or 1:2 for electrolyte analysis.

Sampling frequency

At what interval such samples could be safely repeated in an individual patient would depend upon the health of the animal and its environmental and nutritional management. Hirshfeld & Gordon (1965a) found that when 15% of blood volume was taken eight times in eleven days from six healthy *Trachemys scripta*, mean haemoglobin after 14 days was 2.5 g%, as opposed to 6.1 g% in controls, and haematocrit 9.6% as opposed to 19.3%. All animals, however, appeared to survive the experiment!

In a second experiment, turtles were starved during the period following bleeding (Hirshfeld & Gordon 1965b). In contrast to

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controls that received normal nutrition, these animals showed no measurable evidence of subsequent increased erythropoiesis in circulating blood.

Nutter *et al.* (2000) found that a hospitalised female loggerhead *Caretta caretta*, which lost blood during hemiovariosalpingectomy, recovered its PCV from 13% post-operatively to 25% ten weeks later. A Hermann's tortoise *Testudo hermanni*, which presented with a severed prolapsed oviduct, suffered severe blood loss and had a haematocrit of 7%. The low PCV persisted for another ten days after surgery, but climbed to 18% in three weeks (Meyer 2002).

Factors affecting results

A number of factors may affect blood-test results, including sampling site, ambient temperature, gender and stress. Table 7.3 below gives the effects of some variables on clinicopathological parameters.

Sampling site

The choice of sampling site is important and may influence results. The risk of lymph or extravascular fluid dilution of blood varies from site to site (Gottdenker & Jacobson 1995). An abnormally staining background on blood smears should raise suspicion of lymphodilution, although it should be noted that heparinised samples normally have a blue tinge to them.

Blood taken from sinuses may be less well mixed than those taken from the jugular veins, but it may not be safe to assume that jugular samples can always be taken as 'gold standard' either. However, it is probably safe to say that the more cleanly a jugular sample is taken, the more faith may be placed in the results.

Clipped-nail samples are subject to a variety of possible sources of error resulting from tissue maceration (Mader & Rosenthal 1998).

The results of haematological and biochemical measurements in samples taken simultaneously from the jugular vein and caudal venous sinus of ten animals, were compared. (McArthur *et al.* in preparation). Lymphodilution occurred in 40% of tail samples. The remaining 60% of tail samples yielded results similar to those for the jugular sample from that animal.

Although conventional wisdom dictates that tail samples are much more likely to be lymphodiluted than jugular samples, in 20% of animals the PCV measured was actually higher in the tail sample compared to the jugular. This raises the possibility that

Table 7.3 Potential effects of some variables on clinicopathological parameters.

Parameter	Female	Male	Temperate zone summer	Increased temperature	Haemolysis or failure to separate cells	Lympho-dilution	Acute stress
Albumin	Seasonally ↑		↑ in female	↓		↓	
Globulins						↓	
Total protein	Seasonally ↑		↑ in female	↓		↓	
Total calcium	Seasonally ↑		↑ in female			↓	
Phosphate				↓	↑	↓	
Sodium			↓/↑				
Potassium			↑	↓	↑	↓	
Alkaline phosphatase				↑		↓	
LDH					↑	↓	
AST				↑	↑	↓	
ALT				↑		↓	
CK				↓	↑		
Uric acid						↓	
Urea		↑	↓				
Glucose		↑	↑	↑	↓	↓	
Cholesterol	↑				↓	↓	
Ketones							
PCV		↑	↓			↓	↑
WBC			↑			↓	
Heterophils			↑				
Lymphocytes			↑				
Eosinophils			↓				

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lymphodilution may also occur in jugular blood samples. Indeed, in our experience lymph or extravascular fluid is sometimes visibly aspirated from the neck during attempted venepuncture.

When apparent lymphodilution of the tail sample occurred it appeared to affect different parameters to different degrees:

- Urea, GGT, phosphate and calcium were little affected. It is interesting to speculate as to the influence of the molecular properties and physiological roles of these substances—it may be that they are present in similar concentrations in lymph and blood.
- CK and creatinine were unpredictably affected: differences between jugular and tail samples were often present but neither site was consistently higher than the other. Local tissue contamination of the sample may occur.
- PCV, total white cell count (particularly lymphocytes), LDH, uric acid, AP, albumin and globulins were frequently lower in tail samples (often all 30%–50% reduced in parallel), presumably through lymphodilution.
- ALT was significantly reduced in most tail samples by 40%–80%.

In a similar study, Gottdenker & Jacobson (1995) compared samples from the jugular vein with others from the post-occipital venous plexus. They found significant reductions in glucose, potassium, chloride, uric acid, calcium, phosphate, total protein, albumin, globulin, AP, AST, ALT and cholesterol in the post-occipital samples. This was attributed to dilution of the post-occipital samples with lymph or extravascular fluid.

Temperature

The homeostatic mechanisms of reptiles are more significantly affected by environmental changes (including temperature) than those of mammals and birds. Amongst numerous illustrations of this point are the findings of Lutz & Dunbar-Cooper (1987). They showed that a group of apparently healthy North American loggerhead turtles had a mean haematocrit of 15% whilst, two years later, also in December, turtles from the same site had a mean haematocrit of 35%. This kind of phenomenon presents a severe challenge to clinicians attempting to assess an individual patient when limited 'normal' values are available.

Anderson & Wack (1996) measured blood parameters in 29 New Guinea snapping turtles (*Elseya novaeguineae*) maintained at 24.5°C and 30°C (high and low ends of their preferred optimum temperature zone). The changes set out in Tables 7.4 and 7.5 were significant.

Other investigators have found, in contrast, that temperature did not affect total protein in *Chrysemys picta* (Stinner & Waddle 1988).

Table 7.4 Clinicopathological parameters significantly higher in animals maintained at 24°C than at 30°C (Anderson & Wack 1996).

Parameter	Range at 24.5°C	Range at 30°C
CK (IU/l)	99–5644	60–1275
Albumin (g/dl)	2.0–3.0	1.8–2.3
Total protein (g/dl)	6.2–7.7	5.8–7.3
Potassium (mEq/l)	2.8–4.1	2.1–3.6
Phosphorus (mg/dl)	2.7–6.4	1.4–5.3

Table 7.5 Clinicopathological parameters significantly higher in animals maintained at 30°C than at 24°C (Anderson & Wack 1996).

Parameter	Range at 24.5°C	Range at 30°C
Glucose (mg/dl)	52–147	53–201
AP (IU/l)	39–163	48–157
AST (IU/l)	28–326	46–435
ALT (IU/l)	1–7	2–10
Carbon dioxide (mEq/l)	12–24	15–26
Plasma chloride (mEq/l)	86–97	91–96

Table 7.6 Blood parameters differing significantly between genders (Anderson & Wack 1996).

Parameter		Male	Female
Higher in males	Haemoglobin (g/dl)	7.2–12.1	5.9–12.4
	PCV (%)	29–46	26–43
Higher in females	Cholesterol (mg/dl)	55–308	104–385
	Calcium (mg/dl)	9.6–12.9	9.9–22.2
	Bilirubin (mg/dl)	0–0.3	0–0.4

Gender

Anderson & Wack (1996) also looked at possible gender differences. Their significant findings are shown in Table 7.6.

Male African hingebacks (*Kinixys erosa*) have significantly higher PCV (mean 34% as opposed to 26%) and haemoglobin concentrations than females (Oyewale *et al.* 1998). However, in green turtles (*Chelonia mydas*) (Bolten & Bjorndal 1992) and pancake tortoises (*Malacochersus tornieri*) (Raphael *et al.* 1994a), no significant difference between the sexes was demonstrated in these respects. Changes in albumin, calcium and cholesterol levels, related to female reproductive activity, are discussed below under the individual parameters. Such changes are frequently seasonal. Raphael *et al.* (1994) demonstrated higher glucose levels in male pancake tortoises *Malacochersus tornieri* than in females (which seemed less active). Lutz & Dunbar-Cooper (1987) noted that exceptionally high urea levels were seen in some male loggerhead turtles *Caretta caretta*.

Greek and spur-thighed tortoises (*Testudo hermanni* and *T. graeca*) which were regularly sampled over a period of a year showed significant gender-specific differences in triglycerides, cholesterol and calcium. Triglyceride levels in females were significantly higher throughout the seasons, whereas cholesterol levels were only significantly higher in summer and winter. Calcium levels were significantly higher in females in summer and fall (Meyer: in preparation).

Stress

The effects of acute stressors (such as capture, handling, venepuncture) may not be equivalent to that of chronic stressors (such as inappropriate environment or nutrition). The influence of such variables on clinicopathological parameters is largely unknown. Lutz & Dunbar-Cooper (1987) found that, when loggerhead sea turtles *Caretta caretta* were restrained on deck for

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three hours, their haematocrit increased by an average of 10.4%. By four hours values were 4.8% above immediate post-capture levels and by five hours they had subsided again to below baseline. It was hypothesised that marine turtles may have the ability to sequester and release red cells into the circulation in response to stimuli.

HAEMATOLOGY

White blood cells

Total white cell count

Due to the difficulty in distinguishing between cell classes there are, at present, no established automated methods for performing white cell counts on reptilian blood samples. Lawton & Divers (1999) found that buffy-coat depth, visually assessed, was an unreliable measure. Manual counting is therefore necessary.

Unfortunately, results may vary depending on the technique used and the skill of the individual technician. Our surgery has used two commercial laboratories—both of which handle reptile blood samples on a daily basis. The mean total white cell count for patients in our hospital according to one is 3.7×10^9/l (n = 21) and from the other 8.8×10^9/l (n = 21) for samples with a broadly similar seasonal distribution.

Both direct (using Natt and Herrick's or Rees and Ecker's staining solutions) and semi-direct techniques (such as the eosinophil Unopette method which requires a manual differential count) have been used. In one marine turtle study, counts taken using the Natt-Herrick method exceeded Unopette method counts by an average of 38.5% (Arnold 1994). Raskin (2000) recommended that the Unopette method be avoided. Natt and Herrick's solution has a short shelf-life and must be prepared in-house. Rees and Ecker's solution, in contrast, is stable for several months or more. Lawton (2000) also noted that when using Natt and Herrick's solution the darkly-stained leucocytes can be difficult to differentiate from immature erythrocytes.

The manual white cell counting technique using Rees and Ecker's solution (MacDonald 1999) is described below:

Apparatus required:

- microscope;
- Neubauer counting chamber;
- pipette(s) capable of accurately measuring 20 µl and 1.6 ml;
- Rees & Ecker's solution (in the United Kingdom available from Leeds Veterinary Laboratories);
- damp chamber (sealed box containing soaked gauze or similar);
- mixing roller.

Technique:

- dilute 20 µl of whole heparinised blood in 1.6 ml of Rees & Ecker's solution or ammonium oxalate;
- leave to stand 15 minutes;
- load the counting chamber with the mixture;
- leave 15 minutes in a damp chamber;
- count all the white cells (which stain blue) in each of the broadly-hatched corner zones (each of which is made up of 4×4 smaller cells);
- divide total count by five to give the total number of white cells $\times 10^9$/l.

In practice it can be difficult consistently to distinguish between white cells, thrombocytes and immature erythrocytes—especially at lower magnification. Thrombocyte morphology varies with species and is sometimes very close to that of lymphocytes. It is helpful to have the benefit of having already identified any individual peculiarities of the sample when performing the differential count.

In the author's experience, it is easier to achieve reproducible results by including thrombocytes in the Neubauer chamber count and then performing a correction based on a differential count on a smear. This is an approach also advocated by Raskin (2000).

Differential white cell count

Numerous stains are acceptable. Rapid stains have obvious advantages and are adequate. More time-consuming alternatives may facilitate differentiation between the cytoplasm of the various leucocyte classes (Frye 1991a; Alleman *et al.* 1996). May-Grunwald-Giemsa may make it easier to differentiate between thrombocytes and lymphocytes (Muro *et al.* 1998b). It has been suggested that rapid stains do not reliably stain basophils and that May-Grunwald-Giemsa is preferable—at least in marine turtles (Whitaker & Krum 1999). Wright's stain (Eosin-Methylene blue, Merck) has very good staining properties and allows good differentiation of the different leucocytes and thrombocytes. The cytoplasm of thrombocytes remains translucent compared with the bluish cytoplasm of lymphocytes.

Leucocyte identification in reptiles is very much an evolving area (summarised by Montali 1988). The date of publication is an important consideration when relying upon information in the literature. In particular, azurophils have not been distinguished in the past by most authors.

The chief problems, in this author's experience, arise in separating monocytes from immature or atypical lymphocytes and heterophils from eosinophils. Severely-ill animals may also have very immature circulating cells which cannot with any certainty be assigned to a specific lineage. Garner *et al.* (1996) did not find circulating myeloblasts in a small number of desert tortoises (*Gopherus agassizii*) from which bone marrow was also examined. Red cell precursors are routinely present in peripheral blood smears and may account for most of the unidentified blast cells. Sudanophilia might prove a useful identification criterion since this is a consistent feature of the erythrocyte series.

An illustration of the problems of interpretation can be seen in a comparison of average monocyte counts from two reputable commercial laboratories which deal with reptilian blood samples on a daily basis: laboratory A (n = 11) produced a mean monocyte count of 0.057×10^9/l where laboratory B (n = 18) counted 0.39×10^9/l. As discussed above, laboratory A also has a mean total white cell count 50% lower. An eightfold difference in monocyte counts arouses suspicion that certain cells are being allocated to different lineages by different haematologists.

Similarly, in Fig. 7.18 below, Lawrence & Hawkey (1986) described eosinophil counts in Mediterranean tortoises varying seasonally between a low of 0.05×10^9/l in winter and a high of 1.3×10^9/l in autumn, whereas our laboratories A and B found no eosinophils at all in 24/30 samples taken throughout the year.

Variations in interpretation are an obstacle to progress. It can sometimes be difficult to know whether a change in differential white cell count is due to real change in patient status or simply

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Table 7.7 Morphological characteristics of blood cells in chelonians stained with a rapid Romanowsky stain (RapiDiff II, Diachem International).

Cell type	Size and shape	Nucleus	Cytoplasm	Comments
Erythrocyte (Fig. 7.1)	10–18 µm ellipsoid with smooth margins (Saint Girons 1970)	central; ellipsoid	pinkish; uniform; often with inclusions or vacuolation (Table 7.11)	Mitosis & binucleation (Fig. 7.1) may occur, particularly with inflammation, post-hibernation or anorexia. Spindle- or sickle-shaped cells may be more common in animals with evidence of haemolysis (RW: personal observation).
Immature erythrocyte (Fig. 7.2)	variably sized; rounder and less regular than erythrocyte	central; larger; rounder; less regular	darker, bluer and greyer than mature cells; Sudanophilic (white cells are not)	Multiple pathways of red-cell maturation may exist. Some precursors are smaller than mature erythrocytes *et al.* Generally distinguishable from white cells by clear, deep blue cytoplasm.
Heterophil (Fig. 7.3)	large with irregular margins and sometimes pseudopodia	round to oval, usually eccentric; often non-segmented (segmentation possibly commoner in animals with active inflammation, but also not uncommon in apparently healthy animals)	deep, dull orange refractile variably-shaped granules; often rods or spindles; toxic changes: bluish cytoplasm, abnormal (purplish) granules, vacuoles (Fig. 7.4)	Garner *et al.* (1996) described myeloblasts as having 'a moderate amount of agranular, lightly basophilic cytoplasm and a large, central to slightly eccentric ovoid, vesicular nucleus with a large nucleolus'. In their small series of animals, circulating myeloblasts were not detected.
Eosinophil (Fig. 7.5)	11–17 µm large; rounded (Saint Girons 1970)	variable shape; often pushed against cell margin; may be lobed	pale orange; round granules	When unsure, heterophils and eosinophils are sometimes lumped together in differential counts. This at least eliminates uncertainty.
Basophil (Fig. 7.6)	8–15 µm small; spherical (Saint Girons 1970)	non-lobed; often obscured by granules	variable numbers of dark-blue granules; important to distinguish between a basophil and a toxic heterophil where the granules become dark and round	
Lymphocyte (Figs 7.7 & 7.8)	variable size; often small; irregularly round	eccentric; irregularly round; usually non-lobed but occasionally indented even in normal animals; relatively pale staining	scant cytoplasm; pale blue and clear; may contain phagocytosed particles and sometimes small eosinophilic granules; may have small pseudopodia and peripheral granules in some preparations—this may be artefactual	Lymphocytes are often moulded to adjacent cells. Immature lymphocytes have more cytoplasm and nucleoli. They are similar to mammalian lymphocytes.
Plasmacyte (Fig. 7.9)	similar to lymphocyte; often slightly larger	non-lobed; eccentric	more than lymphocyte; deep blue; finely granular with pale, peri-nuclear halo	
Monocyte (Figs 7.10 & 7.11)	diameter generally 1–1.5 × erythrocyte length; generally smooth outline	variable; often oval with indent (bean- or B-shaped); may have a single large nucleolus	pale blue cytoplasm; often vacuolated or 'foamy'; finely granular; may contain phagocytosed material	Monocytes, being large, are often dragged to the extremities of the smear. It is important to examine all areas to avoid missing them.

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Table 7.7 (*cont'd*)

Cell type	Size and shape	Nucleus	Cytoplasm	Comments
Azurophil or Neutrophil or Neutrophilic Azurophil (Figs 7.11–7.15) (Frye 1991)	smaller than monocytes; diameter often about $^{2}/_{3}$ erythrocyte length; often have pseudopodia and an irregular outline	non-segmented; irregularly round to ovoid; indented or bi-lobed; coarse chromatin; may have a single small nucleolus	bluish to lavender (darker than monocyte); variable vacuolation; often with low numbers of dull, lavender-staining (azurophilic) granules of various sizes plus phagocytosed particles; chelonian azurophils rarely exhibit the striking purple cytoplasm of iguana azurophils	The situation with regard to identification is clouded by varied identification and classification by different authors. Their existence in chelonians is not agreed upon. Raskin (2000) states 'this cell is unique to iguanas and many species of snake' while Alleman *et al.* (1992) describe a variant of monocyte in the desert tortoise with azurophilic granules. Garner *et al.* (1996) found that azurophils 'occasionally [contained] low numbers of small cytoplasmic metachromatic granules'. This author (RW) feels that in most animals, where sufficient cell numbers are examined, distinct azurophil and monocyte populations can be distinguished, on the basis of criteria described in this table.
Thrombocyte (Figs 7.8 & 7.16)	small; oval	oval; central; much darker staining than lymphocytes; thrombocyte nuclei in some species, e.g. *Testudo horsfieldi* and *Cuora* spp. have a pronounced 'crease'–a pale line across the nucleus	very pale blue	Often clumped, may be mistaken for lymphocytes. Where thrombocytes are being recruited into the red-cell pool intermediate cells may be apparent.

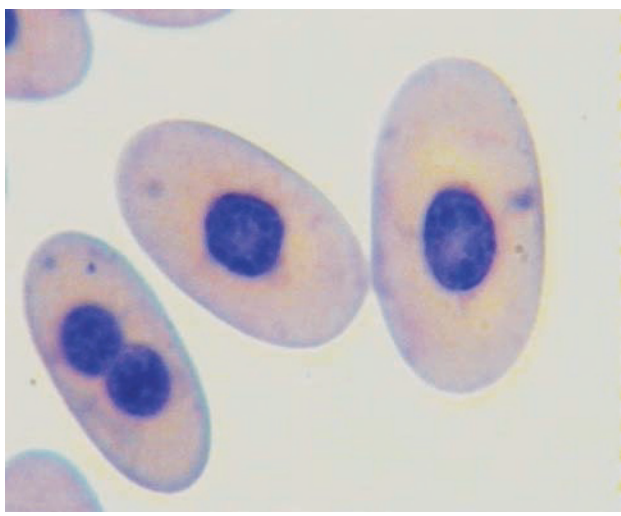

Fig. 7.1 Three erythrocytes in a blood smear from a Horsfield's tortoise (*Testudo horsfieldi*). Rapi-Diff staining; approximately × 1000 magnification.

The left-hand cell is binucleate. This is not uncommon in healthy animals. Retention of the nucleus gives reptilian erythrocytes great versatility. They may undergo mitosis in circulation.

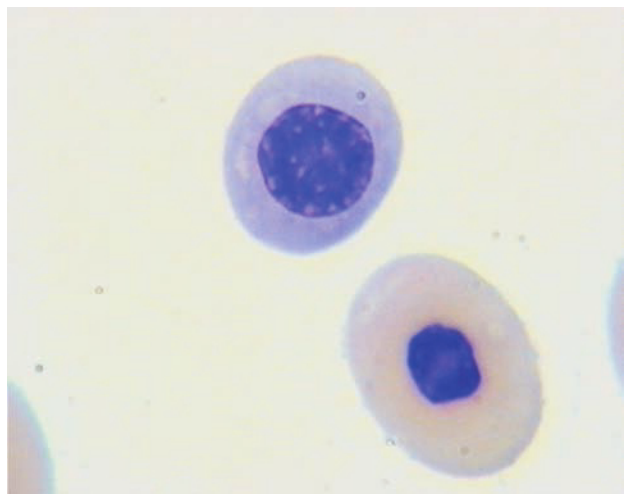

Fig. 7.2 An **immature erythrocyte** (top) and a mature red blood cell (bottom) from a healthy red-eared slider (*Trachemys scripta*). Rapi-Diff staining; approximately × 1000 magnification.

The immature cell is rounder, with a larger nucleus and more basophilic cytoplasm. Multiple pathways of erythrocyte genesis seem to exist in chelonians. Immature cells may be larger than mature ones or smaller, as here.

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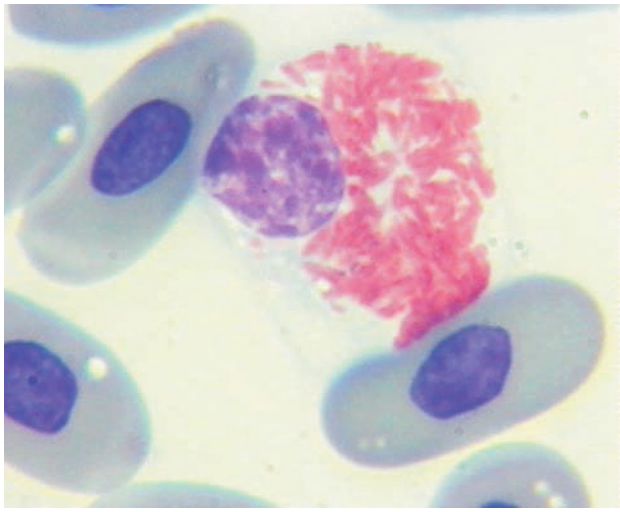

Fig. 7.3 A heterophil in the same preparation as Fig. 7.5.
The cytoplasmic granules are spindle-shaped and dull red—the exact appearance may vary considerably. The nucleus may be multi-lobed.
Approximately × 1000 magnification.

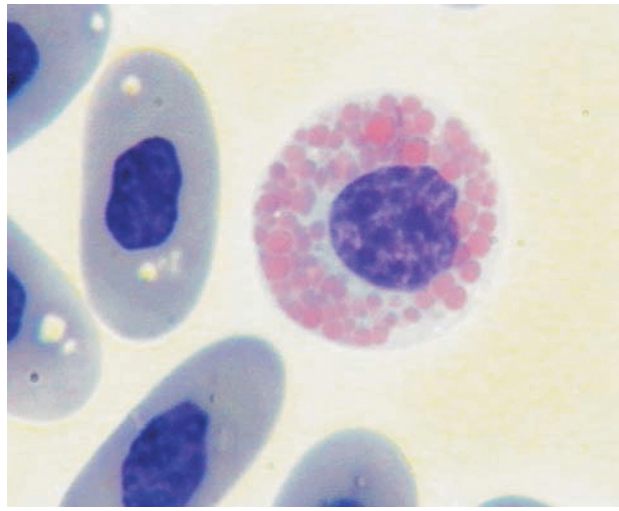

Fig. 7.5 An eosinophil and erythrocytes from a *Testudo whitei* with stomatitis and septicaemia. Rapi-Diff. As seen under approximately ×1000 magnification.
The erythrocytes exhibit cytoplasmic vacuolation—a common finding, particularly in animals with viral disease. The eosinophil nucleus is non-lobed and eccentric while the cytoplasmic granules are round and quite bright orange.

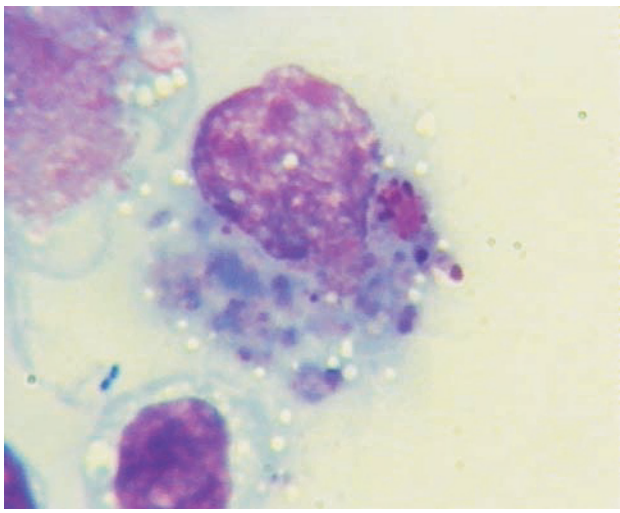

Fig. 7.4 A toxic heterophil in the same preparation as Fig. 7.5.
This cell has largely degranulated (a phenomenon which commonly occurs as an artefact in stored blood samples). The cytoplasm contains 'angry' looking basophilic bodies. A bipolar, rod-shaped bacterium is present lower left.
Approximately × 1000 magnification.

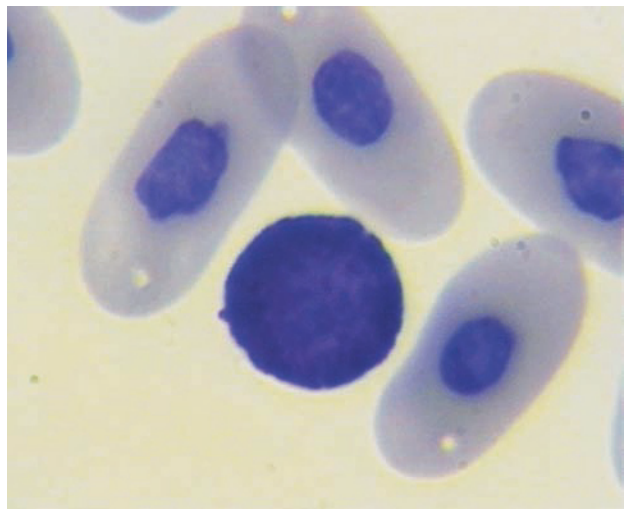

Fig. 7.6 A basophil from an anorexic *Geochelone sulcata*. Rapi-Diff staining; approximately × 1000 magnification.
The nucleus is almost obscured by basophilic granules. In some species (typically freshwater turtles) high basophil counts are a normal finding.

the result of the application of different cell identification criteria. A case can be made for simplification in the interests of consistency —as in the 'lumping' together of heterophils and eosinophils under the heading 'acidophils' (Cooper 1999).

Care must be taken to examine all areas of the smear in order to get a representative sample of white cells. Monocytes, and to a lesser extent heterophils, are often dragged to the edges of the smear and concentrated there by virtue of their large size and can easily be underestimated.

Apparent myeloproliferative disease has been described in an immature turtle (*Pseudemys elegans*) (Frye & Carney 1972). Blood smears from this animal were characterised by a high proportion of blast cells with frequent mitotic figures. Both myelocytic and erythroid cell lines were involved. The distinction between neoplastic and reactive changes is not easy to draw in such cases. Post-mortem examination also revealed a bronchopneumonia in this case, which might be interpreted as either a sequel to or a cause of such haematological findings.

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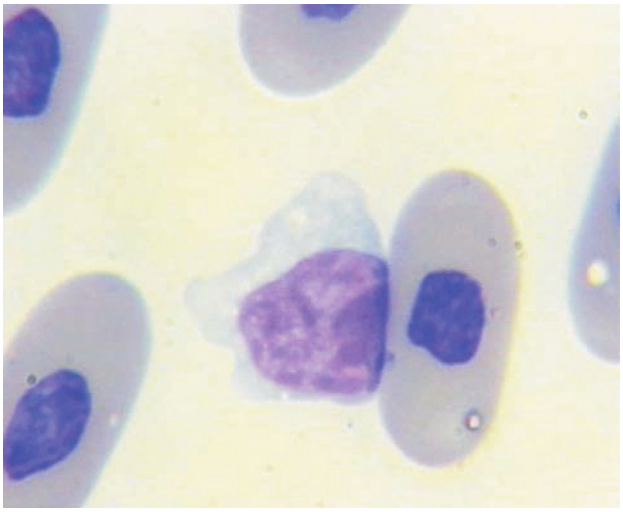

Fig. 7.7 A lymphocyte and erythrocytes from a Mediterranean tortoise (*Testudo*) Rapi-Diff staining; approximately × 1000 magnification.

The erythrocyte immediately to the right of it contains an artefactual refractile inclusion. Lymphocytes have relatively pale-staining, heterogeneous nuclear chromatin and a variable amount of clear, pale blue cytoplasm. Small pseudopodia may be apparent and blue-staining cytoplasmic granules are sometimes present

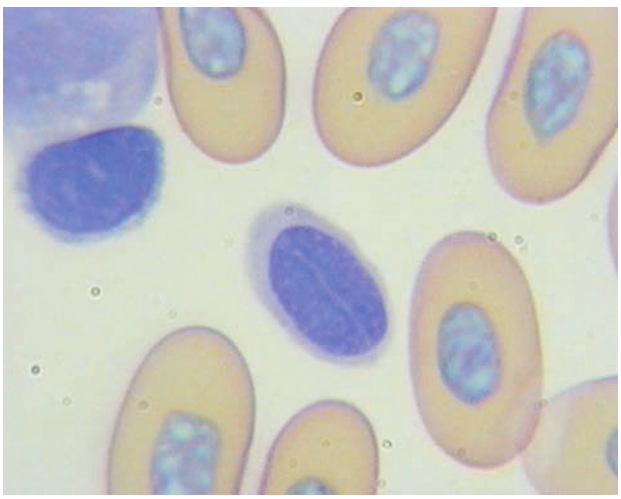

Fig. 7.8 A lymphocyte (upper left) and thrombocyte (lower right), amid erythrocytes, from a Horsfield's tortoise (*Testudo horsfieldi*). Approximately × 1000 magnification.

These cell types can be difficult to distinguish. The lymphocyte has an eccentric nucleus which stains less intensely. Thrombocytes are often clumped together, while lymphocytes are often moulded to adjacent cells (in this case to a monocyte).

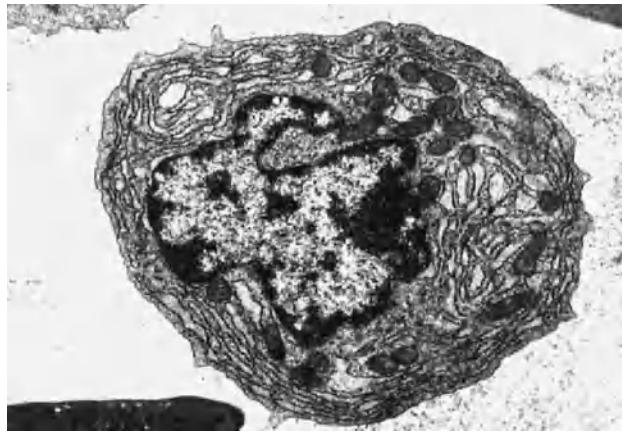

Fig. 7.9 Electron micrograph of a reptilian plasmacyte, stained with phosophotungstic acid, after cold glutaraldehyde-osmium tetroxide fixation. × 6300 original magnification.
(Courtesy of Dr. Fredric L. Frye)

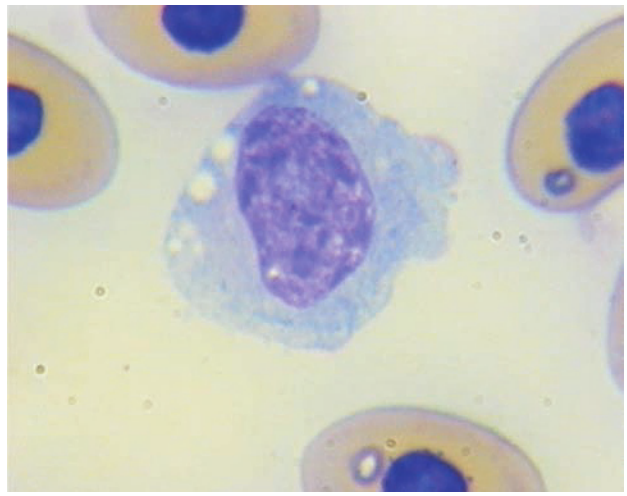

Fig. 7.10 A **monocyte** from an anorexic *Geochelone sulcata*. Rapi-Diff staining; approximately × 1000 magnification.

These large cells are frequently dragged to the edge of the smear during preparation. These areas should therefore be examined specifically to avoid underestimation of monocytes.

Fig. 7.11 A monocyte (bottom right) and an azurophil (top left) from a Horsfield's tortoise (*Testudo horsfieldi*) with stomatitis. Rapi-Diff staining; approximately × 1000 magnification.

The monocyte is larger, with a smooth outline, a B-shaped nucleus, and pale blue, finely-granular cytoplasm.

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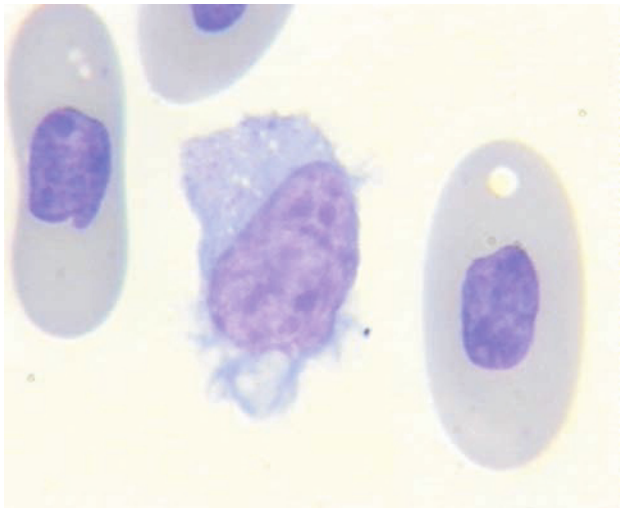

Fig. 7.12 An azurophil and erythrocytes from a *Testudo whitei* with stomatitis and septicaemia. Rapi-Diff staining; approximately × 1000 magnification.

Azurophils are consistently smaller than monocytes with more basophilic cytoplasm. Lavender-coloured cytoplasmic granules are usually present and vacuolation is common. The cell outline is irregular.

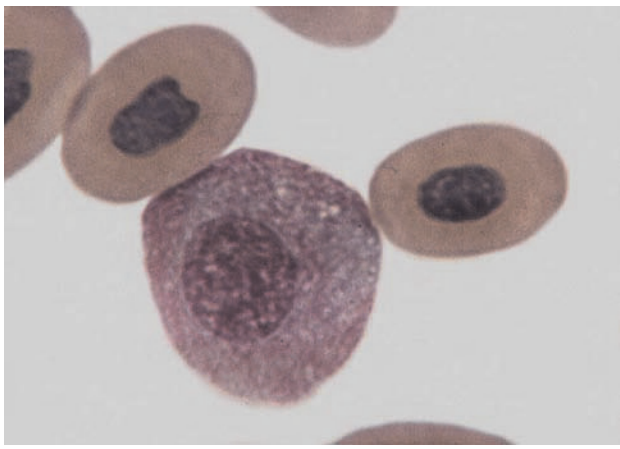

Fig. 7.13 A normal **azurophil** from an African leopard tortoise (*Geochelone pardalis*). Jenner-Giemsa staining; × 400 original magnification. (Courtesy of Dr. Fredric L. Frye)

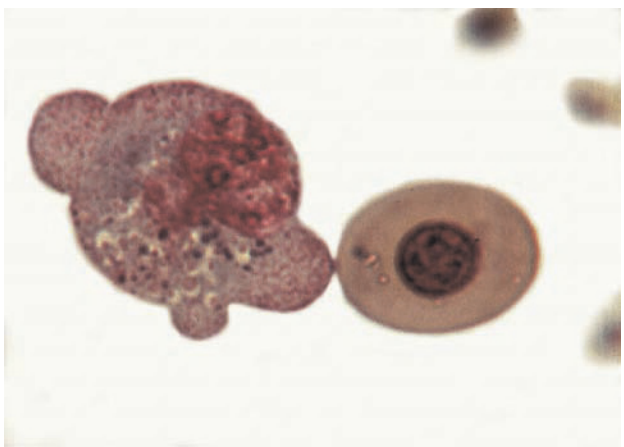

Fig. 7.14 **Azurophil** with multiple pseudopodia (African leopard tortoise, *Geochelone pardalis*). Jenner-Giemsa staining; × 400 original magnification. (Courtesy of Dr. Fredric L. Frye)

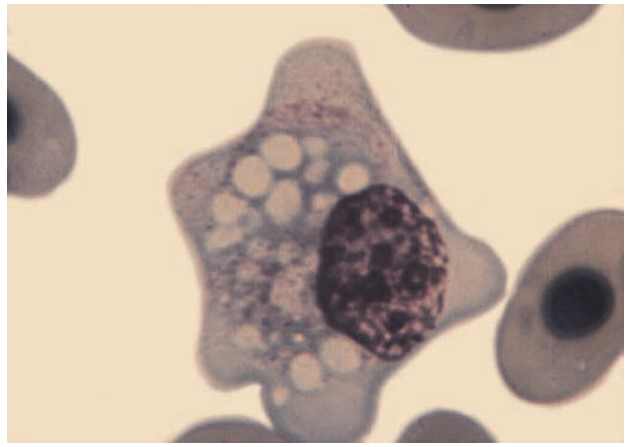

Fig. 7.15 A **toxic azurophil**, exhibiting multiple, clear intracytoplasmic vacuoles (African leopard tortoise, *Geochelone pardalis*). Jenner-Giemsa staining; × 400 original magnification. (Courtesy of Dr. Fredric L. Frye)

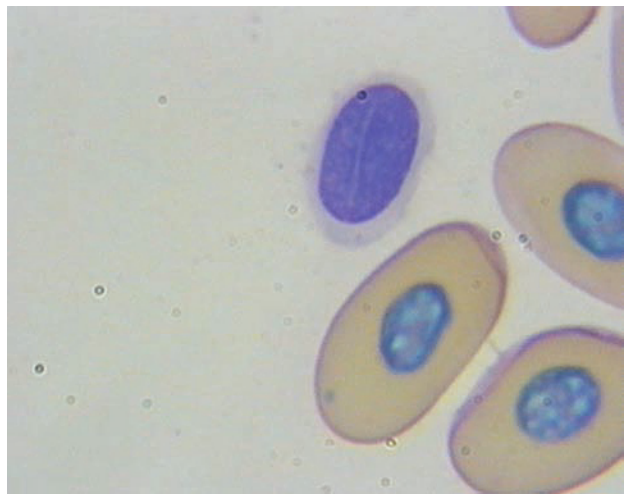

Fig. 7.16 A **thrombocyte** (and several erythrocytes) in a blood smear from a Horsfield's tortoise (*Testudo horsfieldi*). Rapi-Diff staining; approximately × 1000 magnification.

Thrombocytes are smaller than erythrocytes and ellipsoid. They have pale grey cytoplasm and a central nucleus with dark, condensed chromatin. In some species, as here, the nucleus has a characteristic linear feature.

'Normal' haematological values

The whole issue of 'normals' for captive reptiles is a vexed question, since it is difficult to define what constitutes a normal animal and normal management conditions. It is also not particularly meaningful to compare results from a potentially sick, long-term captive animal with data from wild populations. Normal values can be more usefully applied when their origin with respect to species, age, sex, season, sample site, nutrition and other management conditions are known.

Marked seasonal variations in red and white blood cell parameters occur in chelonians from temperate zones—especially if hibernation occurs (Figs 7.17–7.18) (Table 7.8). For this reason it is of limited value to give an all-encompassing normal range.

Blood becomes more concentrated during hibernation as water reserves are depleted. The marked drop in December was

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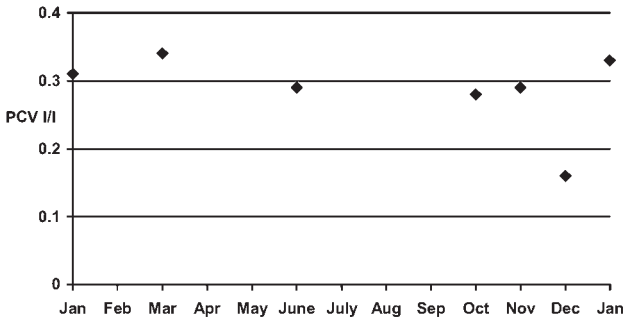

Fig. 7.17 Seasonal changes in mean packed cell volume (PCV) in eighteen healthy, long-term captive, winter-hibernated Mediterranean tortoises, using clipped-nail samples (Lawrence & Hawkey 1986).

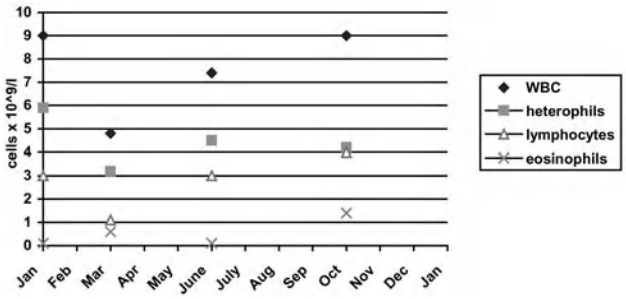

Fig. 7.18 Seasonal changes in mean white blood cell counts in ten healthy, long-term captive, winter-hibernated *Testudo graeca,* using clipped-nail samples (azurophils not recognised) (Lawrence & Hawkey 1986).

a dramatic feature in the year covered by the data presented in Fig. 7.17. It was suggested that this might be due to sequestration of red cells in liver and or spleen, since there appears to be no parallel dilution of other blood components (Lawrence 1987a). Further work to investigate this interesting phenomenon over multiple annual cycles and using a variety of sampling sites might be rewarding. It is unknown to what extent this drop might occur in non-hibernated animals.

In some species gender-related differences in PCV are seen (Table 7.6).

Table 7.8 Seasonal variation in differential white blood cell count in healthy, winter-hibernated, long-term captive *Testudo graeca* and *T. hermanni*, using clipped-nail samples (azurophils and plasmacytes not recognised) (Lawrence & Hawkey 1986).

Cell type	January (n = 10)	March (n = 8)	June (n =6)	October (n = 8)
Heterophils (10^9/l)	5.8 ± 1.6	3.1 ± 1.9	4.3 ± 2.0	3.9 ± 1.7
Lymphocytes (10^9/l)	3.0 ± 1.4	1.1 ± 0.5	2.9 ± 1.0	3.7 ± 1.1
Monocytes (10^9/l)	0.02 ± 0.05	0.06 ± 0.12	0.1 ± 0.1	0.08 ± 0.2
Eosinophils (10^9/l)	0.05 ± 0.1	0.46 ± 0.61	0.06 ± 0.13	1.3 ± 1.38

Again, it is not known to what extent winter decreases in lymphocytes and heterophils and increases in eosinophils are seen in non-hibernated animals.

Table 7.9 below lists some parameters for other species.

In healthy marine turtles, Whitaker & Krum (1999) reported haematocrits of 30.1 ± 3.0 (n = 15) for rehabilitated Kemp's Ridley (*Lepidochelys kempii*) and 22.0 ± 5.3 (n = 11) for captive juvenile loggerheads (*Caretta caretta*) weighing less than 2 kg. Bolten & Bjorndal (1992) reported a mean packed cell volume of 35.2 (range 26–42) for healthy, wild, juvenile green turtles (*Chelonia mydas*).

Marked species differences occur. George (1997) reported that neutrophils (azurophils) have not been found at all in Kemp's Ridley (*Lepidochelys kempii*) while they are common in loggerheads (*Caretta caretta*). Our (RW & SM) chelonian patients (predominantly Mediterranean *Testudo* spp.) do not follow the pattern of 'typical' healthy reptile differential white cell counts described by Frye (1986). Whereas Frye suggests that a typical azurophil count for a healthy reptile would be 3%–7%, our patients, with a variety of illnesses, have far fewer (one third having none, the remainder ranging up to 0.95×10^9/l). Frye's basophil count of 20%–25% is very much higher than would be typical in our hospital (mean count 0.14×10^9/l), and the same is true for eosinophils (7%–20% as opposed to being present in only 6/30 samples taken by us). Figures for monocytes (0.5%–3%), lymphocytes (15%–89%), heterophils (20%–40%) and plasmacytes (0.2%–0.5%) are broadly comparable to our own.

Table 7.9 Haematological parameters in healthy chelonians of other species.

Parameter	Radiated tortoise *Testudo radiata*	Desert tortoise *Gopherus agassizii*	Loggerhead turtle *Caretta caretta*
Haematocrit (%)	31 ± 7.4	23–37	35 (range 28–48)
Haemoglobin(g/l)	6.7 ± 1.5		
White blood cell count	4.3 ± 1		
Lymphocytes (10^9/l)	1.6 ± 0.12	25–50	
Heterophils (10^9/l)	2.0 ± 0.1	35–60	
Monocytes (10^9/l)	0.15 ± 0.02	0–4	
Basophils (10^9/l)	0.34 ± 0.04	2–15	
Eosinophils (10^9/l)	0.18 ± 0.03	0–4	
Azurophils	not recognised	0–3	

Sources:
Radiated tortoise: Marks & Citino (1990)—ten long-term captive animals, axillary vein samples, summer
Desert tortoise: Rosskopf (1982)—300 captive animals, clipped-nail samples
Loggerhead turtle: Lutz & Dunbar-Cooper (1987)—103 wild animals, cervical venous samples throughout year in the United States

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The proportion of erythroblasts has potential diagnostic significance since it might be used to distinguish regenerative from non-regenerative anaemias. In practice, however, this parameter is often so variable in healthy animals that it is difficult to draw conclusions from a single value in one sick individual. In healthy *Trachemys scripta*, Hirshfeld & Gordon (1965a) reported that bleeding led to an increase in erythroblasts from 0.4%–2.4% of circulating red cells, and in reticulocytes from 0–6.9% by 14 days after bleeding.

In a variety of sick chelonians treated in our hospital, manual thrombocyte counts ranged from $1–29 \times 10^9$/l (Wilkinson & McArthur: unpublished data). Raskin (2000) reported that counts derived by automated nucleated cell counting in combination with a manual 1000 cell differential gave a range of $5–30 \times 10^9$/l for four healthy desert tortoises (*Gopherus agassizii*)—an exceptionally close correlation.

Interpretation of haematology results

It has been suggested (Mader 1996a) that, in view of the variability of reptile haematological parameters, only counts at least double normal values should be considered significant. Table 7.10 below gives some differential diagnoses based on haematology results.

Haemoparasites

For many red-cell inclusion types our understanding is based on very little hard fact and much speculation. At present we have to accept that, for individual cases, many such agents cannot be identified with confidence under light microscopy. Designations such as *Pirhemocyton*, *Chelonoplasma* or *Nuttallia* currently have little meaning. The published work on these phenomena in chelonians is based on the morphological appearance of what

Table 7.10 Possible significance of haematological findings in chelonians (bold type denotes phenomena supported by published evidence or observed at Holly House Veterinary Surgery, Leeds, United Kingdom; others are speculative).

Heterophilia	**Seasonal** (more in summer) **Infection and inflammation** Stress **Trauma** Neoplasia Lack of heterophilia does not rule out infectious disease. We have seen numerous cases with severe viral or bacterial disease (pneumonia, abscess/fibriscesses, and cellulitis) where the heterophil count was normal or low. Toxic changes are an important finding.
Heteropaenia	**Infection**: Mean acidophil (heterophils plus eosinophils) count in the acute phase of a stomatitis-blepharitis epidemic amongst recently-imported *Testudo horsfieldi* (n = 23) was 1.3×10^9/l (McArthur & Wilkinson: unpublished data). After clinical recovery (in early spring) this had risen to 2.8×10^9/l. This relative heteropaenia was not associated with an adverse prognosis in this group—all recovered with treatment.
Eosinophilia	**Seasonal** (more in winter, especially if hibernating) **Parasitism** (Rosskopf 1982): In our experience in the United Kingdom it is uncommon for parasitised chelonians to show eosinophilia. In a later paper, Rosskopf (2000) reports that eosinophilia is most consistent with parasitism in desert tortoises, water turtles and box turtles and that correlation between eosinophil count and haemoparasite load is variable. **Other inflammation** (one case of cellulitis)
Lymphocytosis	**Seasonal** (more in summer) **Females and young** may have higher counts **Inflammation and healing** Parasitism Leukaemia
Lymphopaenia	**Sick tortoises** (12/26 at Holly House): Mean lymphocyte count in the acute phase of a stomatitis-blepharitis epidemic amongst recently-imported *Testudo horsfieldi* (n = 23) was 6.2×10^9/l. After clinical recovery (in early spring) this had risen to 11.3×10^9/l. **Tail vein samples** **Anorexia/malnutrition** of any cause **Hibernation** Lymphoproliferative disease
Monocytosis	**Acute or chronic infection:** Mean monocyte count in the acute phase of a stomatitis-blepharitis epidemic amongst recently-imported *Testudo horsfieldi* (n = 23) was 2.68×10^9/l. After clinical recovery (in early spring) this had declined to 1.2×10^9/l. **Inflammation** **Green turtle fibropapillomatosis** (Work & Balazs 1999) **Chronic renal failure** (Wilkinson & McArthur: unpublished data) The frequency with which monocytosis is diagnosed depends very much on the reference range used. In the United Kingdom, reference ranges of up to 2.25×10^9/l have been used by commercial laboratories—this is higher than we have ever recorded at our hospital in a Mediterranean *Testudo*. We have seen numerous chelonians with chronic inflammatory disease (e.g. abscess/fibriscesses, pneumonia) with monocyte counts below 0.3×10^9/l. Higher counts ($0.3–0.92 \times 10^9$/l) were associated with pre-ovulatory ovarian stasis (POOS), post-hibernation anorexia of unknown cause, stomatitis (Herpesvirus infection) and chronic renal failure.

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Table 7.10 (*cont'd*)

Plasmacytosis	**Infection or inflammation**
Azurophilia	**Infection or inflammation**: In our hospital, high counts (>0.2 × 10^9/l) are uncommon. Such cases have included post-hibernation anorexia of unknown cause, wounds, blepharitis (possibly Herpesvirus), upper respiratory tract inflammation and cloacal prolapse. However, in the acute phase of a stomatitis-blepharitis epidemic amongst recently-imported *Testudo horsfieldi* (n = 23) mean azurophil count was 0.53 × 10^9/l. After clinical recovery (in early spring) this had actually risen to 1.3 × 10^9/l. This is an interesting finding since the monocyte counts demonstrated the opposite trend. This might suggest some difference in the roles played by azurophils and monocytes. Interpretation of counts has been complicated by problems of classification and identification in the past. As a result some 'normal' values may be misleading.
Basophilia	**Normal:** High counts are normal in red-eared sliders (*Trachemys scripta*), snapping turtles (*Chelydra serpentina*) (Rosskopf 2000) and possibly other species. It is possible to speculate that this might be associated with higher levels of parasitism in aquatic and semi-aquatic chelonians (e.g. Martin 1972). **Immunological response**: The highest count we have seen in a Mediterranean tortoise was 1.63 × 10^9/l associated with upper respiratory tract inflammation. Several other individuals with stomatitis and/or RNS have had counts in the range 0.28–0.42 × 10^9/l. Rosskopf (2000) reports basophilia in chronically-ill desert tortoises. **Intestinal parasitism** (Rosskopf 2000) **Haemogregarine infection** (personal observation)
Anaemia	**Lymphodilution** of sample should be considered **PCV may be lower in females and juveniles** (Holly House data; Whitaker & Krum 1999) **Season:** In healthy Mediterranean *Testudo* tortoises, haematocrit reaches a maximum at the end of hibernation. In sick tortoises with post-hibernation anorexia it is often low, despite clinicopathological evidence of dehydration. **Poor nutritional and environmental conditions** **Blood loss** **Erythrolysis** (autoimmune or haemoparasitic) **Anaemia of chronic disease** (pneumonia, RNS, renal disease in cases seen at this author's surgery [RJW]): Garner *et al.* (1996) found that Mycoplasma-infected desert tortoises (*Gopherus agassizii*) had lower PCVs than healthy animals. Similarly, Work & Balazs (1999) found that green turtles (*Chelonia mydas*) afflicted with fibropapillomatosis had haematocrits which declined with increasing severity of lesions. Leukaemia Myelophthisis (might occur with lymphoproliferative disease) 15/52 sick tortoises were judged anaemic at the author's clinic (RJW). Very high circulating erythroblast counts may suggest regeneration. Hirshfeld & Gordon (1965a) produced a peak erythroblast response of 23% of circulating red cells after bleeding.
Thrombocytopaenia	**Anaemia:** In severe anaemia, thrombocytes may be recruited into the erythrocyte pool.

might ultimately prove to be a heterogeneous population of red cell inclusions that cannot be correlated with confidence with any clinical manifestation of disease. Electron microscopic (EM) studies may be the key to further advances. EM pictures of *Sauroplasma* (to which *Chelonoplasma* bears some morphological similarities under light microscopy) have recently been published and provide strong evidence that this is indeed a protozoan (Alberts *et al.* 1998). Anticoagulated blood samples for transmission electron microscopy (TEM) should generally be preserved in glutaraldehyde or formalin, although this should be discussed with the laboratory concerned.

Table 7.11 summarises the current state of our knowledge of haemoparasites.

In summary, the pathogenic significance of many haemoparasites is unclear. Many of them seem non-pathogenic in healthy animals. Given the embryonic state of our knowledge, however, this should not be assumed to be invariably true. Little is known about treatment of these infections. For a fuller account the reader is referred to Telford (1984).

BLOOD BIOCHEMISTRY

Blood biochemistry values

Because reptiles exert less control over their homeostatic mechanisms than birds and mammals their 'normal' ranges are often wider and in many species are subject to marked seasonal variation. As discussed under haematology, the validity of normal values depends upon knowledge of the circumstances of their source (species, age, sex, season, sample site, nutrition and other management conditions). Differences in technique employed by individual laboratories are also important. This is particularly so when assaying enzymes (AP, LDH, AST etc.) for which critical details of incubation temperature, substrate and buffer will vary.

The best diagnostic values are obtained where reference values for that individual have already been established when the individual was healthy. Unfortunately, such opportunities are generally limited.

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Table 7.11 Haemoparasites and red cell inclusions in chelonians.

Inclusion	Morphology with Romanowsky rapid stain	Significance	Prevalence in captive animals in the United Kingdom
Howell Jolly bodies	Small, round bodies within cytoplasm staining as nucleus	Nuclear remnants of unknown significance	Uncommon
Degenerate organelles? (Fig. 7.19)	Small basophilic spots or rings	Alleman *et al.* (1992) considered such findings likely organelles	Possibly not uncommon
Heinz bodies	Irregular refractile bodies	Denatured haemoglobin may suggest oxidant toxins (onions, brassicas); artefacts of processing	Artefactual refractile inclusions are common in our laboratory. Reasons for this are unclear
Herpesvirus and possibly other viruses	Circular, clear intra-erythrocytic vacuoles approximately 2–4 µm in diameter (may be described as 'viral pools')	Described by Frye (1991c) in two turtles in which herpes-virus-like particles were found. May not be specific to herpesvirus. Similar findings are typical of 'boid inclusion body disease' in snakes. Iridovirus diseases also cause red-cell inclusions in other species (Frye 1999).	Common finding. Strongly associated with pathological signs (e.g. RNS, stomatitis) and clinicopathological evidence of viral disease. However, vacuolation was not a feature in the acute phase of a stomatitis-blepharitis epidemic amongst recently-imported *Testudo horsfieldi* (McArthur & Wilkinson: unpublished data)
Haemogregarines (Fig. 7.20) Protozoa, including genera *Haemogregarina* and *Hepatozoon,* affecting chelonians (Telford 2000)	Intracellular gametes (~10 µm); banana-shaped or ovoid; in erythrocytes or white cells; often displace cell nucleus; paler than erythrocyte cytoplasm, with blue-purple granules	Usually mildly- or non-pathogenic (Telford 1984); indirect life-cycle (arthropod and leech vectors–including mites); not present in marine turtles; transmission from one host species to another may occur	3/55 patients studied; probably much commoner in aquatic or semi-aquatic species
Pirhemocyton (Figs 7.21 and 7.22)	Small, round, blue-purple 'dots' within erythrocytes (Fig. 7.21). We have seen both *Geochelone pardalis* and *G. sulcata* with relatively large, 4 µm *Pirhemocyton*-like inclusions (Fig. 7.22). In Mediterranean tortoises they are typically 1–2 µm. The cytoplasm of affected cells often also contains non-staining, circular 'albuminoid bodies' the significance of which is unknown.	Although *Pirhemocyton*-like inclusions are often reported, there is only one published account of *Pirhemocyton* in chelonians (Acholonu 1974). This report describes the appearance under light microscopy of red-cell inclusions in freshwater turtles and draws no firm conclusion as to their identity. In other reptiles, an iridovirus may be responsible (Stehbens & Johnston 1966; Telford 1984).	16/57 patients at Holly House; in one anorexic juvenile *Geochelone sulcata* an intra-erythrocytic *Pirhemocyton*-like organism was the only finding of significance. Many affected animals are apparently healthy.
Chelonoplasma (Fig. 7.22)	Intra-erythrocytic 'signet rings'; 2 µm rings with one or more pigmented granules on the periphery (occasionally centrally); morphologically similar to *Sauroplasma* and *Serpentoplasma* which may be piroplasmid protozoa (Alberts *et al.* 1998)	This is a poorly-defined entity in chelonians (Frye 1991a). There are no well-documented reports in the literature. Pathogenicity is unknown, although some affected animals were unwell (Frye 1999: personal communication).	Red cell inclusions have been seen in a group of 14 severely ill *Geochelone pardalis* in our surgery. These were tentatively identified as *Sauroplasma*-like (Telford 1999). A herpesvirus was also isolated from these animals.
Piroplasms (Fig. 7.23)	*Nuttallia* is described from a single case in *Testudo campanulata.* The parasite takes a variety of forms within the red cell but often has four bunched nuclei (Carpano 1939). Morphologically similar organisms were seen in a *Testudo graeca* (Peirce & Castleman 1974).	These are two apparent haemoparasites, described on the basis of light microscopic appearance, the nature of which remains unknown; of unknown pathogenicity (although the *Nuttallia* tortoise was dead!)	Uncommon

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Table 7.11 (*cont'd*)

Inclusion	Morphology with Romanowsky rapid stain	Significance	Prevalence in captive animals in the United Kingdom
Haemoproteus	No circulating schizonts (asexual reproductive phase)—unlike *Plasmodium*; young gametocytes appear as small oval rings (1 µm); mature gametocytes at poles of cell become slightly larger than cell nucleus (up to 10 µm); 'potato-shaped' with pigment granules and/or vacuoles (excellent illustrations in Lainson & Naiff 1998)	Unknown (Lainson & Naiff (1998); possibly mildly or non-pathogenic; might cause anaemia; arthropod vectors; reported from African, Indian, Australian and North American chelonians (Telford 1984)	Two cases
Plasmodium	Not reported in chelonians by Ayala (1978), Telford (1984 & 2000) or Keymer (1981) although Frye (1991a) includes a plate depicting a parasite described as *Plasmodium* in a leopard tortoise (*Geochelone pardalis*). There are other anecdotal reports of *Plasmodium* in tortoises. However, this author is not aware of any published accounts. The latest twist is a report by MacDonald (2000) who discusses preliminary findings with the use of the 'Immunocapture *Plasmodium* lactate dehydrogenase (LDH) assay' in reptiles. *Plasmodium* LDH (pLDH) can be distinguished with this test from human LDH. Although it has not been validated for reptilian host species, MacDonald found good correlation, in a small number of individuals, between results of this assay and findings on examination of conventional Giemsa pH 7.2-stained blood smears. One of nine *Testudo graeca* evaluated in this trial was found to have both 'visible evidence of *Plasmodial* infection' in blood smears and a positive pLDH result. Further details were not given.		
Trypanosomes	Free-swimming in plasma; single long flagellum attached to body by undulating membrane	No symptoms reported in chelonians (Telford 1984)	None seen to date in our hospital. Reports from Chelydridae, Testudinae, Chelydidae (Telford 1984)
Filariids	Microfilariae in blood	*Cardionema* adults inhabit the of chelonian hearts; no symptoms were reported by Frank (1981)	No cases
Bacteraemia	Extracellular cocci or bacilli on blood smear	Consistent with severe primary or secondary bacterial disease	Not uncommon in severely-ill animals
Spirochaetaemia	Thin 'coils' visible in plasma on blood smears	Pathogenic; associated with malaise and death (Frye & Williams 1995)	Frye & Williams' case involved Asian box turtles (*Cuora* spp.) in transit

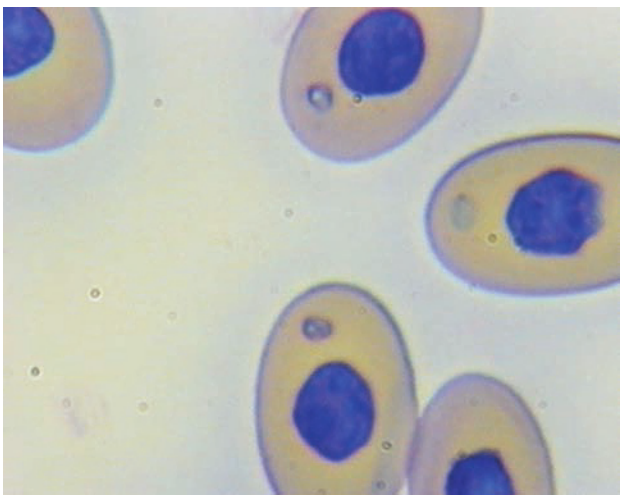

Fig. 7.19 Erythrocyte cytoplasmic inclusions in a blood smear from an anorexic, mature *Geochelone sulcata*.

Irregular annular inclusions such as these are often seen in various species. Electron microscopy of such samples suggests that these are degenerate organelles, but viruses (e.g. *Pirhemocyton*) can appear similar under the light microscope.

Approximately × 1000 magnification.

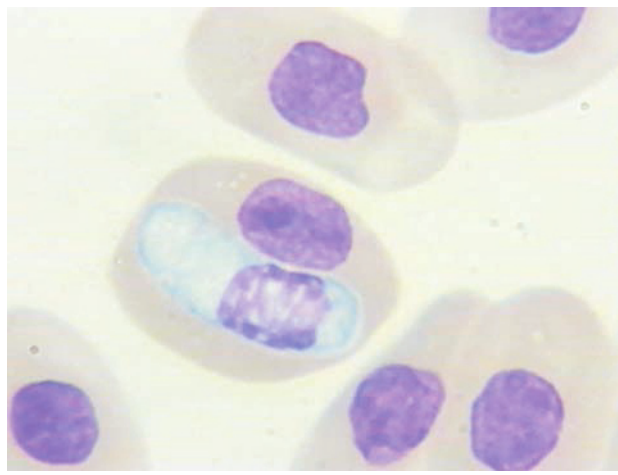

Fig. 7.20 An intra-erythrocytic Haemogregarinid in a blood smear from an Asian box turtle (Cuora). Rapi-Diff staining; approximately × 1000 magnification.

The precise morphology of haemogregarines is variable. They are typically banana- or potato-shaped.

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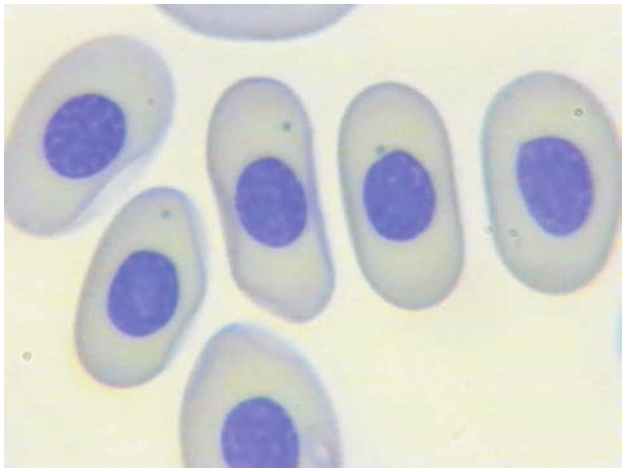

Fig. 7.21 Erythrocyte cytoplasmic inclusions in a blood smear from a Horsfield's tortoise (*Testudo horsfieldi*) with stomatitis. Rapi-Diff staining; approximately × 1000 magnification.

Small, punctate, basophilic inclusions such as these are common and are usually described as *Pirhemocyton*. Their identity is unknown.

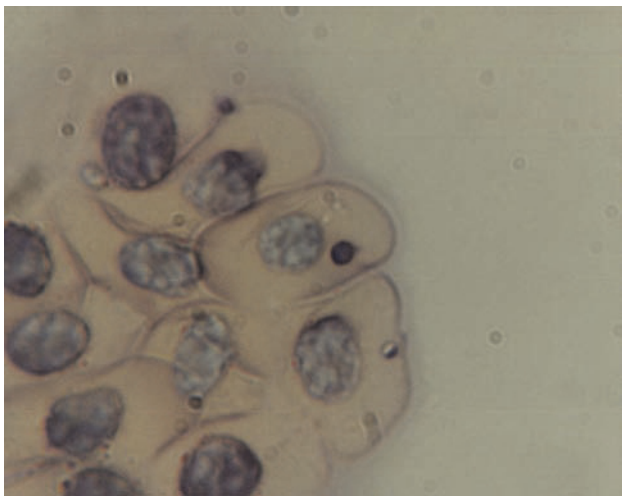

Fig. 7.22 Erythrocyte inclusions. Rapi-Diff staining; approximately × 1000 magnification.

The upper of the two most right-hand cells contains a circular basophilic inclusion which is Pirhemocyton-like. The cell below it contains a 'signet-ring' body similar to those tentatively described as *Chelonoplasma*. Again, the identity and significance of these inclusions are unknown.

The plasma of many healthy chelonians is yellow-orange coloured. This may be the result of plant pigments (Nakamura 1980) and should not necessarily be interpreted as indicating excessive or abnormal haemoglobin degradation (i.e. jaundice).

Tables 7.12 and 7.12a give some blood biochemistry values for apparently healthy chelonians. *These values have been taken from small numbers of animals, and may differ significantly from those of other individuals of the same species where age, gender, season, sample site, nutrition or management conditions are different.*

Table 7.13 shows the seasonal variation in electrolyte and urea concentrations in *Testudo hermanni*.

No information is given by the authors as to the number or gender of tortoises involved or whether or not they were captive

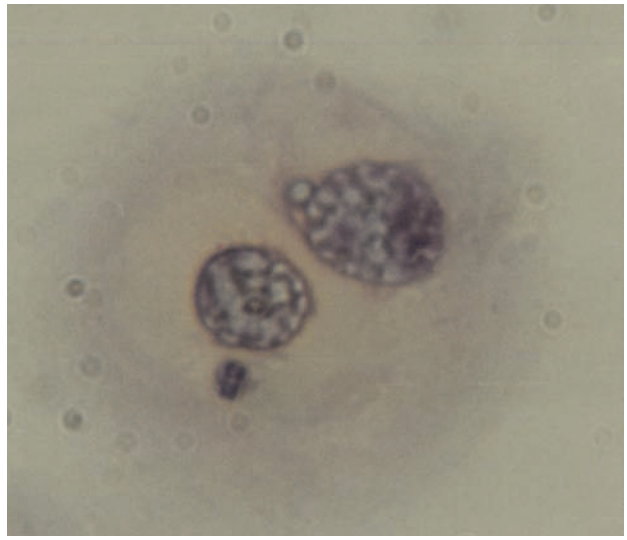

Fig. 7.23 An apparent haemoparasite (with four blue-staining components) within an erythrocyte from an anorexic juvenile *Geochelone sulcata*. Rapi-Diff staining; approximately × 1000 magnification.

The identity of this red-cell inclusion is unknown. It bears some resemblance to an organism described under the name *Nuttallia* (Carpano 1939).

animals. As discussed below, female tortoises may show seasonal calcium changes. Aldred (1939) found that osmotic pressure in captive tortoises varied widely from individual to individual even when all were sampled at the same time of year. The variability of osmotic pressure of blood has important implications for fluid therapy (see Therapeutics).

Table 7.14 shows the seasonal changes in electrolyte and urea concentrations in loggerhead sea turtles (*Caretta caretta*).

Sodium and chloride levels were lowest in the coldest months of the year — a phenomenon which has also been noted in hibernating freshwater turtles. This is exactly the opposite of the increases seen in hibernating terrestrial *Testudo hermanni* in the preceding table. The reason for this is unknown. Potassium levels tend to be higher in the warmer months for both species.

Table 7.15 shows the seasonal variation in blood glucose in Mediterranean tortoises.

In desert tortoises (*Gopherus agassizii*) Rosskopf (1982) reported normal values of 1.7–8.4 mmol/l.

Interpretation of results

This section deals with the significance of changes that may be seen in serum biochemistry values. For ease of use, the parameters have been arranged in alphabetical order.

Some conversion factors are:

- Inorganic phosphate: mmol/l × 3.1 = mg/dl
- Calcium: mmol/l × 4 = mg/dl
- Uric acid: µmol/l = mg/dl × 58

Albumin

Low albumin values have been seen at Holly House in animals with anorexia, malnutrition, stomatitis, intestinal parasitism and other enteropathies, and when there has been lymphodilution. It has been hypothesised that it might be seen in hepatic disease

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Table 7.12 Blood biochemistry values from apparently healthy chelonians (SI units).

	Mediterranean tortoise *Testudo hermanni*	Mediterranean tortoise *Testudo hermanni/graeca*	Desert tortoise *Gopherus agassizii*	Leopard tortoise *Geochelone pardalis*	Pancake tortoise *Malacochersus tornieri*	African hingeback *Kinixys erosa*	Kemp's Ridley *Lepidochelys kempii*	Loggerhead sea turtle *Caretta caretta*	Green sea turtle *Chelonia mydas*	Aldabran giant tortoise *Dipsochelys gigantea*
Total protein g/l	31–54	13–38	22–50	32–49	24–41	61 ± 0.44	32 ± 6	20 ± 8	26–69	5–38
Albumin g/l		5–18		12–22	12–21	28 ± 0.44	16 ± 3	10 ± 2.6	6–21	10–17
Calcium mmol/l	2.7–3.5	2.0–2.9	2.3–4.3	2.35–3.5	2.4–4.6	2.1 ± 0.04	1.57 ± 0.32	1.8 ± 0.2	0.4–2.6	1.5–4.5
Phosphate mmol/l	1.7–3.3	0.45–1.7		0.7–1.32	0.7–1.4	1.48 ± 0.1	2.9 ± 0.58	1.7 ± 1.1	1.2–3.6	0.3–1.3
Uric acid µmol/l	125–577	35–244	130–547	71–95	52–541			41 ± 18	29–206	58–360
Creatinine µmol/l	<26	14–25	9–36	79–218	9–27	140 ± 2.65			26–79	0–220
AST U/l	19–103	18–222			83–534		161 ± 43	123 ± 38	31–389	
LDH U/l	161–473	89–269	25–250				6298 ± 5284	108 ± 63	48–342	
GGT U/l	<10	0–1.0				2.58 ± 0.23				
AlkP U/l	196–425	61–211		30–125		157 ± 2.7	465 ± 306	74 ± 37	13–95	
Cholesterol mmol/l					0.7–6.1	1.86 ± 0.04			1.9–9.5	0.3–3.9
Triglyceride mmol/l						0.62 ± 0.02			0.49–4.7	
Potassium mmol/l	4.5–5.0		2.2–4.5	3.8–5.1	4.2–6.1	4.87 ± 0.06	4.1 ± 0.8	4.6 ± 0.4	4.1–6.9	
Sodium mmol/l	130–144		130–157	128–145	111–146	122 ± 0.7	150 ± 4	157 ± 1	157–183	
Chloride mmol/l	96–115				83–116	98 ± 0.19	116 ± 4	121 ± 10	100–130	
T4 U/l		10–24.8								

(although animals seen by us with histopathological evidence of hepatic lipidosis have had normal albumin levels), blood loss or renal disease.

Hypoalbuminaemia appears to be commoner in our hospital in immature animals. Whether this is just because they are often sicker or because healthy immature chelonians always have lower blood protein is not known. The values given by Whitaker & Krum (1999) for immature loggerheads (*Caretta caretta*) are very much lower than for adult chelonians of other species in Table 7.12.

Lawrence (1987a) found that in summer females have albumin levels on average 30% higher than males, probably due to vitellogenesis (yolk-formation). This may account for higher summer protein levels seen in non-hibernating *Trionyx spiniferus* (Seidel 1974), although gender differences were not discussed by Seidel. In Mediterranean tortoises in northern England this effect may be seen from March through to July (Holly House data). Hence Harcourt-Brown's (1994: personal communication) total protein figures for September range only up to 38 g/l while Göbel & Spörle (1992), who did not give the season of sampling, recorded values up to 54 g/l—a figure similar to reference values used by many commercial laboratories in the United Kingdom.

In contrast, hibernating *Trachemys scripta* and *Terrapene carolina* have higher protein levels in winter. This may be attributed to resulting haemoconcentration (Hutton & Goodnight 1957)–again, gender differences were not discussed.

Raised values are also seen in dehydration.

Alkaline phosphatase (AlkPhos)

There is some alkaline phosphatase present in kidney and intestine, but serum levels suggest that primary sources may be tissues that have not been investigated to date (possibly bone and/or reproductive organs) (Ramsay & Dotson 1995).

Relatively high levels were found in a leopard tortoise (*Geochelone pardalis*) with metabolic bone disease (Raiti & Haramati 1997). Hypovitaminosis D has also been reported as causing elevations in plasma alkaline phosphatase in mammals (Lian *et al.* 1987).

In our hospital the highest levels have been seen in immature animals (765–1157 IU/l) and in females with pre-ovulatory follicular stasis (666–1272 IU/l). Levels over 450 IU/l have not been seen in other patients presented to us.

Alanine aminotransferase (ALT)

ALT is present in the kidneys (250 units/g) with smaller amounts in the liver (40 units/g), according to Ramsay & Dotson (1995). Wetzel & Wagner (1998) found low levels in a variety of tissues

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Table 7.12a Blood biochemistry values from apparently healthy chelonians (US metric units).

	Mediterranean tortoise *Testudo hermanni*	Mediterranean tortoise *Testudo hermanni/graeca*	Desert tortoise *Gopherus agassizii*	Leopard tortoise *Geochelone pardalis*	Pancake tortoise *Malacochersus tornieri*	African Hinge-back *Kinixys erosa*	Kemp's Ridley *Lepidochelys kempii*	Loggerhead sea turtle *Caretta caretta*	Green sea turtle *Chelonians mydas*	Aldabran giant tortoise *Dipsochelys gigantea*
Total protein g/dl	3.1–5.4	1.3–3.8	2.2–5.0	3.2–4.9	2.4–4.1	6.1 ± 0.044	3.2 ± 0.66	2.0 ± 0.8	2.6–6.9	0.5–3.8
Albumin g/dl		0.5–1.8		1.2–2.2	1.2–2.1	2.8 ± 0.044	1.6 ± 0.3	1.0 ± 0.26	0.6–2.1	1.0–1.7
Calcium mg/dl	10.8–14.0	8.0–11.6	9.2–17.2	9.4–14.0	9.6–18.4	8.4 ± 0.2	6.3 ± 1.3	7.2 ± 0.8	1.6–10.4	6–18
Phosphate mg/dl	5.1–9.9	1.4–5.1		2.1–4.0	2.1–4.2	4.4 ± 0.3	8.7 ± 1.74	5.1 ± 3.3	3.6–10.8	0.9–3.9
Uric acid mg/dl	2.1–9.8	0.6–4.1	2.2–9.3	1.2–1.6	0.9–9.2			0.7 ± 0.3	0.5–3.5	1.0–6.1
Creatinine mg/dl	<0.3	0.16–0.28	0.1–0.4	0.9–2.5	0.1–0.3	1.6 ± 0.03			0.3–0.9	0–2.5
AST U/l	19–103	18–222			83–534		161 ± 43	123 ± 38	31–389	
LDH U/l	161–473	89–269	25–250				6298 ± 5284	108 ± 63	48–342	
GGT U/l	<10	0–1.0				2.58 ± 0.23				
AlkP U/l	196–425	61–211		30–125		157 ± 2.7	465 ± 306	74 ± 37	13–95	
Cholesterol mg/dl					26–231	71 ± 2			72–361	11–148
Triglyceride mg/dl						55 ± 2			43–413	
Potassium mEq/l	4.5–5.0		2.2–4.5	3.8–5.1	4.2–6.1	4.87 ± 0.06	4.1 ± 0.8	4.6 ± 0.4	4.1–6.9	
Sodium mEq/l	130–144		130–157	128–145	111–146	122 ± 0.7	150 ± 4	157 ± 1	157–183	
Chloride mEq/l	96–115				83–116	98 ± 0.19	116 ± 4	121 ± 10	100–130	
T4 µg/dl		0.8–2.0								

SOURCES:
Testudo hermanni: Göbel & Spörle (1992)—17 captive animals, ulnar vein.
T. hermanni/graeca: Harcourt-Brown (1998: personal communication)—five captive *T. graeca*, seven *T. hermanni*, 6/12 female, tail vein, September.
Gopherus agassizii: Rosskopf (1982)—300 captive American animals, axillary vein or clipped-nail.
Geochelone pardalis: Raiti & Haramati (1997)—eight captive animals, 5/8 female.
Kinixys erosa: Oyewale (1998)—12 recently captured animals, 6/12 female, blood from severed carotid.
Lepidochelys kempii: Whitaker & Krum (1999)—15 healthy rehabilitated turtles at release from aquarium.
Caretta caretta: Whitaker & Krum (1999)—nine captive turtles less than 2 kg body weight.
Chelonia mydas: Bolten & Bjorndal (1992)—100 wild juvenile animals (carapace length 248–679 mm).
Malacochersus tornieri: Raphael *et al.* (1994)—27 wild animals, occipital plexus, blood possibly taken during breeding season.
Geochelone gigantea: Ghebremeskel *et al.* (1991)—44 wild animals on Curieuse Island, tail vein, January–February.

from iguanas. ALT values may be artificially low in samples taken from the tail (Holly House data).

In our experience, ALT is rarely raised, even in the presence of glomerulonephritis. Juvenile animals sampled by us have had very much higher plasma ALT concentrations than adults (9–15 IU/l as opposed to 1–6 IU/l) but small numbers of individuals are involved.

Amylase

Wetzel & Wagner (1998) found high concentrations of amylase only in iguana pancreas, and plasma levels were variable, even in healthy animals.

Aspartate aminotransferase (AST)

Ramsay & Dotson (1995) assessed reptilian enzyme activities in selected tissues. AST was present in significant amounts in liver, kidney and heart (but not skeletal) muscle. Wetzel & Wagner (1998) also found AST to be relatively non-organ-specific.

Page *et al.* (1988) found that an injection of succinyl choline and intravenous catheter placement consistently induced elevations in plasma AST.

In our experience, raised levels of this enzyme are often present in the blood of animals that are sick with a variety of conditions (e.g. herpesvirus infection, glomerulonephritis, wounds, stomatitis, intussusception and septic arthritis). In 5/7 animals with

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Table 7.13 Seasonal changes in electrolyte and urea concentrations in healthy, winter-hibernated *Testudo hermanni* (Gilles-Baillien & Schoffeniels 1965).

	Na^+ mEq/l	K^+ mEq/l	Ca^{++} mEq/l	Cl^- mEq/l	Urea mmol/l	Osmotic Pressure mOsm/l
Jan	156	3.7	2.4	124	31	349
Feb	161	3.0	5.3	123	38	449
March	157	3.8	5.4	125	34	443
April	167	4.6	4.6	134	103	467
May	129	5.0	4.9	86	37	340
June	105	4.3	1.5	66	26	258
July	115	4.5	4.53	94	4	290
Aug	136	4.8	5.5	108	12	322
Sept	136	4.1	4.9	99	11	338
Oct	138	4.2	4.8	110	22	343
Nov	141	3.0	5.2	99	21	349
Dec	155	3.9	6.3	124	31	404

Table 7.14 Seasonal changes in mean electrolyte and urea concentrations in healthy wild loggerhead sea turtles (*Caretta caretta*) in the United States (Lutz & Dunbar-Cooper 1987).

	Na^+ mmol/l	K^+ mmol/l	Ca^{++} mmol/l	Cl^- mmol/l	Urea mmol/l	Osmotic Pressure mOsm/l
Dec	150	3.7	1.1	109	11	315
Jan	138	3.3	1.4	105	6.2	301
Feb	129	3.05	1.2	110	4.6	300
March	142	3.3	1.0	108	5.0	324
April	141	3.6	1.0	112	15.5	340
May	139	3.5		112	9.2	334
June	139	3.5	0.9	103	2.3	305
July	143	3.9	1.1	114	5.7	329
Aug	139		1.6	121	6.8	327
Sept	145	3.8	1.4	121	7.8	332
Nov	162	4.2	2.2	107	9.4	330

Table 7.15 Seasonal variation in blood glucose in winter-hibernated long-term captive Mediterranean tortoises (data from 18 animals) (Lawrence 1987).

	Jan	Feb	Mar	Apr	May	Aug	Oct	Dec
Mean blood glucose (mmol/l)	0.6	0.6	1.2	13.3	6.4	1.5	0.8	0.6

AST levels over 200 IU/l the creatine kinase (CK) concentration was also over 1000 IU/l. Ramsay and Dotson's work would suggest that these animals might have cardiac muscle damage. Whether this is really the case is unknown. If it were the case, it would represent a previously unrecognised incidence of heart pathology in chelonians.

Where CK is not raised, the likelihood is that AST elevations are of hepatic or renal origin.

Beta-hydroxybutyrate

This ketone is thought to be the best indicator of ketogenesis in reptiles (Christopher *et al.* 1994). These authors found that, in desert tortoises (*Gopherus agassizii*), plasma levels varied from 0.4–0.75 mmol/l in times of significant rainfall (and food availability), but increased to 2.0 mmol/l after two months of drought. In contrast, ketogenesis did not appear to be important during hibernation.

This parameter may be a useful measure of health status in animals presented without a known history and provides an objective measure of energy balance in all patients. Urine levels are also potentially of use.

Bile salts

The diagnostic value of bile salt concentrations remains to be proven although this parameter seems to be useful in birds (Carpenter *et al.* 1996). However, test results obtained with avian test standards could lead to completely erroneous results. Chelonians bile salts are of a very specific 27-atom chemical structure which is not to be found in any other vertebrate (Skoczylas 1978).

Anderson & Wack (1996) found that in New Guinea snapping turtles (*Elseya novaeguineae*) bile acid levels measured in serum were significantly higher (range 0–50 mg/dl) than those in plasma (0–44 mg/dl). Gender and temperature (24°C cf. 30°C) did not affect results.

In unfed reptiles, normal values are usually below 60 µmol/l (Divers 1998: personal communication), although this may depend upon the assay technique. In our chelonian patients, with a cross section of disorders, levels varied from 0–15 µmol/l. However, too little is known about the liver function or histopathological state of the liver in most of these animals to draw any conclusions as to the value of the test. In one leopard tortoise (*Geochelone pardalis*) with histopathological evidence of hepatitis the serum bile salt concentration was 1.2 µmol/l.

Biliverdin

Biliverdin may be the primary haemoglobin degradation product in chelonians (With 1968) and might prove a valuable diagnostic tool. At present, however, no assay is readily available.

The plasma of many healthy chelonians (including our *Testudo* patients) is normally yellow-orange coloured. This may be the result of carotenoid plant pigments (Nakamura 1980) and should not be interpreted as indicative of abnormal or excessive haemoglobin degradation. Bolten & Bjorndal (1992) speculated that green turtles (*Chelonia mydas*) with colourless plasma had been feeding carnivorously in the open ocean, whereas those with yellow plasma were benthic herbivores.

Calcium (*see also pages 69–70*)

Serum calcium may be raised by 2%–400% (Dessauer 1970) prior to egg laying. It is unclear to what extent this effect is due to protein levels, which also increase at this time and may increase measured calcium levels through binding site availability. The concept of 'corrected calcium' (Finco 1983), or direct measurement of ionised calcium, may prove useful in elucidating this

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situation. Rosol *et al.* (1998) warn that the correction procedure has not been validated with ionised calcium measurements to confirm that it has biological significance. Studies on the direct measurement of ionised calcium are urgently required.

Vitellogenic hypercalcaemia is seen in female Mediterranean tortoises in the United Kingdom between March and July (Holly House data). The highest values yet recorded have been up to 11.4 mmol/l—very close to a 400% increase on the top end of the reference range. A typical March–July concentration for our female patients would be 4–6.5 mmol/l.

We have not seen a proven male tortoise with a plasma calcium level exceeding 3.1 mmol/l or an immature chelonian exceeding 3.6 mmol/l. This suggests that pathological causes of hypercalcaemia are uncommon. Our experience to date does not support the contention that hypercalcaemia is often a feature of chronic renal failure in tortoises. Hypercalcaemia is reported to occur with less common conditions, such as primary hyperparathyroidism, pseudohyperparathyroidism and with osteolytic bone lesions (Frye 1991a).

Sample lipaemia is a potential cause of artefactually high calcium values (Mader & Klaasen 1998). High values might also occur in over-supplemented (calcium, vitamin D) animals and in animals tube-fed with diets such as Complan.

Low values occur with deficiencies of vitamin D, ultraviolet light or dietary calcium, for example, in captive loggerhead turtles (*Caretta caretta*) with clinically-apparent nutritional secondary hyperparathyroidism (median calcium 0.65 mmol/l) (George 1997).

In our hospital, calcium levels in general are extremely variable. This may reflect wide variations in nutrition and the inclusion of vitellogenic females. Thirty-six out of 61 sick animals had values judged by the laboratories involved to be outside normal limits.

Cholesterol

Cholesterol is likely to be raised in females during reproductive activity and lowered prior to hibernation in temperate species (Derikson 1976). Jacobson *et al.* (1991b) found that cholesterol levels were higher in sick desert tortoises (*Gopherus agassizii*) than in healthy animals.

Using a wide range of chelonian species, Jackson & Legendre (1967) measured blood cholesterol levels to be between 80–480 mg/dl. Small numbers of individuals were used. The levels were lowest in herbivorous species, such as *Gopherus polyphemus*, and highest in carnivorous Chelydrids.

In our patients, we have recorded blood levels between 1.6–14 mmol/l (n = 25). Females with apparent pre-ovulatory follicular stasis were all in the top half of this range (6–13.2 mmol/l). There did appear to be a tendency for females in general to have higher levels in summer but a larger survey would be more conclusive. Low levels were seen in animals which had been chronically anorexic for various reasons. Of four animals with concentrations below 2.5 mmol/l, two subsequently died.

Creatine kinase (CK) or creatine phosphokinase (CPK)

Ramsay & Dotson (1995) found very high creatine kinase/creatine phosphokinase activity in skeletal and heart muscle with much smaller amounts in kidney. This is in accordance with the findings of Wetzel & Wagner (1998) who considered it to be muscle specific in iguanas.

It may be artificially high in samples taken by cardiocentesis, where venepuncture is traumatic (Page 1988: personal observation) and after injection of solutions causing muscle damage, e.g. some preparations of enrofloxacin. That many elevated values are due to traumatic venepuncture is supported by the finding that when tail and jugular samples, simultaneously taken, are compared, the two results were often grossly different, but sometimes the tail and sometimes the jugular concentration was greater (personal data on file).

Creatinine

Creatinine is currently considered to be a poor indicator of renal function. Neither actively secreted nor reabsorbed by the kidney tubules (Dantzler & Schmidt-Nielsen 1966), creatinine clearance ratio is thus a suitable indicator of glomerular filtration rate (and hence renal function) when plasma levels are artificially elevated by intravenous infusion.

Fibrinogen

It has been suggested that fibrinogen may be a better indicator of infectious disease than total white cell count, but little data is available (see Coagulation Parameters below).

Gamma-glutamyl transferase (GGT)

High concentrations were not found in any of the tissues assessed by Ramsay & Dotson (1995). Kidney concentrations were most significant. All animals investigated at our surgery have had concentrations below 5 IU/l. No pattern was apparent.

Glucose

Marked seasonal variations in blood glucose levels occur in temperate zone chelonians (Table 7.15). Reference ranges used by some laboratories in the United Kingdom do not allow for the normal post-hibernation peak. This may lead to false reports of hyperglycaemia.

The post-hibernation surge does not occur in depleted animals: this is an important factor in post-hibernation anorexia. Most of our chronically sick patients have April–May glucose levels of 3–6 mmol/l, whereas acutely ill animals in spring may still have concentrations similar to the 13 mmol/l reported by Lawrence (1987a) (Table 7.15) for healthy animals.

Blood glucose levels are reduced in hepatopathy, anorexia, malnutrition and septicaemia.

Glucose may be elevated by acute stressors (e.g. blood sampling), although this phenomenon has not been investigated. It has been suggested that truly representative samples should be taken through a pre-placed venous catheter.

Possible cases of diabetes mellitus in chelonians with persistent hyperglycaemia have been described (Frye *et al.* 1976; Frye 1991a; Frye 1999). Some of these animals were shown to have pancreatic pathology. The dominant clinical sign was anorexia rather than polydipsia.

Glutamate dehydrogenase (GLDH)

Initial reports suggest that GLDH may prove to be a useful indicator of hepatocellular necrosis in reptiles (Lomas & Waters 2000). It is relatively easy to assay and in other species is a sensitive and specific measure of hepatic damage (Mühlberger & Kraft 1994; Battison *et al.* 1996).

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Lomas & Waters (2000) found that 25/26 apparently healthy captive *Testudo graeca* and *T. kleinmanni* had plasma GLDH below 20 IU/l.

Lactate

Relaxed, captive loggerhead turtles (*Caretta caretta*) had blood lactate levels of 0.2–0.4 mmol/l, whilst those caught in trawl nets showed concentrations of 3.2–16.2 mmol/l (Lutz and Dunbar-Cooper 1987).

Lactate dehydrogenase (LDH)

LDH is present in a wide range of tissues including liver, kidney, skeletal and heart muscle, with smaller amounts in the gut. Levels are often raised in sick animals with tissue damage. Plasma levels in excess of 700 IU/l are associated with, for example, stomatitis, gastrointestinal obstruction, cloacal prolapse, coeliotomy, septic arthritis or renal failure.

Page *et al.* (1988) found that injection of succinyl choline and intravenous catheter placement consistently induced elevations in plasma LDH.

It has been suggested that haemolysis may lead to artificial elevation of LDH (Rosskopf 1982).

Phosphate

If hyperphosphataemia is a finding, consider the possibility of haemolysis of the sample. Phosphate levels may also be higher in young animals (Mader 1996a).

Interpretation of blood phosphate levels is currently clouded by our inability to delineate a 'normal' range because it is impossible to define a 'normal' animal. It has been suggested that most captive animals, in the United Kingdom at least, suffer chronic clinical or subclinical metabolic bone disease through a combination of inadequate ultraviolet exposure and unsuitable diet. On the other hand, we are currently unable to offer firm guidelines for calcium and vitamin D dosages for animals which have good ultraviolet provision, and some of these may be over-supplemented as a result. While this problem remains unsolved it is difficult to gain much information from our results.

In 46 sick animals treated by us, inorganic phosphate levels ranged from 0.62–3.33 mmol/l. One commercial laboratory, using a reference range of 1.3–3.0 mmol/l, judged 14/23 to be hypo- and none to be hyperphosphataemic. Another laboratory, using a reference range of 0.48–1.81 mmol/l, judged 8/23 to be hyper- and none to be hypophosphataemic. Using a combination of 'normal' ranges from Table 7.12 above, they might all have been normal! My personal suspicion is that the top end of the normal range in Mediterranean species should not be higher than 1.7 mmol/l and possibly should be lower. The recently-captured *Kinixys erosa* and the well-managed *Geochelone pardalis* in Table 7.12 above were well below this level. It seems likely that the mixed group of *Testudo hermanni/graeca* would be, if anything, subclinically hyperphosphataemic since they were maintained outdoors in summer in Yorkshire—a low-ultraviolet environment! If this is so, then many of our patients are indeed suffering a degree of nutritional secondary hyperparathyroidism.

Phosphate will be elevated if there is a dietary excess of phosphate relative to calcium, for example in metabolic bone disease. George (1997) reports a group of captive loggerhead turtles (*Caretta caretta*) with clinically apparent nutritional secondary hyperparathyroidism (median phosphate 4.7 mmol/l).

Low levels are seen in anorexia. Bennett (1998c) mentions a syndrome of hypophosphataemia and hypokalaemia which arises when relatively large amounts of nutrient-rich foodstuffs are given to chronically anorexic reptiles. It is suggested that phosphorus and potassium move from blood into cells along with glucose.

The mean phosphate level for patients with uric acid over 1000 µmol/l was 1.40 mmol/l compared to 1.36 mmol/l for animals with a variety of other conditions (McArthur & Wilkinson: unpublished data). This does not support the contention that hyperphosphataemia is characteristic of chronic renal disease in chelonians. However, most of these animals were also chronically anorexic and this will have depressed phosphate levels.

The two animals with the highest plasma phosphate levels in our series (3.33 and 2.43 mmol/l) were both suffering from severe stomatitis and subsequently died. The lowest phosphate levels were seen in a mixed bag of conditions—gastrointestinal obstruction, post-hibernation anorexia/hyperuricaemia and in an apparently healthy, immature leopard tortoise (*Geochelone pardalis*).

In contrast, hyperphosphataemia has been reported with renal disease in iguanas. The Ca:P ratio in these animals was <1 when measured in mg/dl (Boyer 1996a). Of 11 sick tortoises in our hospital with markedly raised uric acid levels, including an animal with histopathologically-demonstrated glomerulonephritis, none had a Ca:P ratio <1, and it remains to be proven whether this ratio is a useful indicator of renal function in Mediterranean tortoises. The precise aetiology of the syndrome described by Boyer (1996a) in *Iguana iguana* and its relevance to chelonians is unclear. In 12 healthy Mediterranean tortoises sampled by Harcourt-Brown (1994: personal communication) in September, the mean Ca:P ratio (as measured in mg/dl) was 3.43 with no significant gender difference.

Potassium

If hyperkalaemia is a finding, consider the possibility of haemolysis of the sample.

Values for our patients varied from 1.99–10.46 mmol/l–a considerable range when compared to the healthy animals in Table 7.12. Gilles-Baillien & Schoffeniels (1965) found that April–August potassium levels (4.3–5.0 mmol/l) were higher in *Testudo hermanni* than autumn–winter levels (3.0–4.1 mmol/l). These were hibernating individuals and it is not known to what extent the winter drop occurs if animals remain active.

Dantzler & Schmidt-Nielson (1966) suggested that blood potassium levels might be difficult to control for animals with high potassium intake and low water availability. Whereas urates precipitate out in the bladder when urine is retained by a dehydrated animal, potassium might equilibrate into the blood along with water. This hypothesis is not altogether borne out by our data. The animals with apparent dehydration (high urea, normal uric acid) had unremarkable potassium concentrations in the range 3.4–5.58 mmol/l (n = 7).

Holmes & McBean (1964) found that sea turtles maintained in fresh water had higher blood potassium levels than those in sea water.

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Bennett (1998c) mentions a syndrome of hypokalaemia and hypophosphataemia which may follow feeding of chronically-anorexic reptiles.

Boyer (1996a) reported hyperkalaemia in *Iguana iguana* with apparent renal failure.

There was a strong correlation between hyperuricaemia and hyperkalaemia (McArthur & Wilkinson: unpublished data). All animals with blood potassium levels over 6.9 mmol/l were hyperuricaemic and all subsequently died. In mammals, blood potassium levels above 7 mmol/l are thought to represent a threat to cardiac function (Michell *et al.* 1989). Some hyperuricaemic patients had potassium values as low as 5.1 mmol/l.

It has been suggested that potassium levels might be reduced in enteropathy or dietary deficiency. The most hypokalaemic (1.99 mmol/l) of our patients was a *Testudo graeca* with yeast enteritis, anorexia and muscle weakness. After two weeks of stomach tubing with liquidised vegetables the potassium level had risen to 2.4 mmol/l. A single dose of 6 mmol/mEq oral potassium (Sando-K, Sandoz) lead to a further increase in plasma potassium to 3.5 mmol/l within 24 hours. One further individual with severe acute stomatitis had a plasma potassium level of 2.7 mmol/l, but the remainder were all above the bottom of the normal range of Gilles-Baillien & Schoffeniels.

Sodium

Considerable seasonal variation occurs (Tables 7.13 and 7.14). In Mediterranean *Testudo*, sodium levels peak in April after hibernation and trough in June after spring fluid intake. In desert tortoises (*Gopherus agassizii*), Christopher *et al.* (1994) also recorded a post-hibernation peak of 143 mmol/l falling to 133 mmol/l by July after rain. Results from sick animals must be interpreted in this light.

In theory, sodium levels may be elevated in conditions of high water loss (diarrhoea) or reduced water intake (inability to drink, non-availability of water). In our patients with a wide range of conditions, levels ranged from 101–163 mmol/l—a close approximation to the 105–167 of Gilles-Baillien & Schoffeniels (1965). The lowest level was recorded in a *Testudo graeca* with renal failure but this was also in June at the time of the expected minimum concentrations. Unseasonably high levels were seen in stomatitis and pneumonia cases—possibly through an inability to drink.

Triglycerides

Triglycerides are likely to be raised in females during reproductive activity and lowered prior to hibernation in temperate species (Derikson 1976).

In our patients, we have measured blood levels between 0.06–7.15 mmol/l (n = 23). The highest level was seen in an anorexic Russian tortoise (*Testudo horsfieldi*) with histopathological evidence of hepatic lipidosis. As with cholesterol, relatively high concentrations (3.4–4.7 mmol/l) were observed in females with pre-ovulatory follicular stasis and females in general may have had higher mean cholesterol. The depression of autumn and winter levels was much more marked for triglycerides than for cholesterol—the mean for samples taken between September–February was 0.9 mmol/l as opposed to 2.1 for March–August.

Thyroid hormones (*see also pages 71–72*)

Very little has been published about thyroid hormone measurements in chelonians. Healthy adult Galapagos tortoises (n = 3) had T4 levels between 1.08–1.54 µg/dl and T3 levels of 33–93 ng/dl (Norton *et al.* 1989). Normal T3 and T4 values are also available for the eastern painted turtle (*Chrysemys picta*) (Sawin *et al.* 1981) and green sea turtle (*Chelonia mydas*) (Licht *et al.* 1985). In Caribbean green turtles, mean T4 levels were consistently around 9 ng/ml throughout the year.

Primary hypothyroidism does not appear to have been conclusively described although Norton *et al.* (1989) discussed this as a possibility in one case of a Galapagos tortoise (*Chelonoidis nigra*). However, animals on diets low in iodine or high in goitrogens have thyroid pathology and clinical signs, including goitre, compatible with hypothyroidism (Frye & Dutra 1974). Galapagos tortoises appear especially susceptible. These patients have low thyroid hormone levels. 'Sick euthyroid' syndrome may be not uncommon: animals with non-thyroid illness have been found to have low or undetectable thyroid hormone levels (Raiti & Haramati 1997). Dynamic thyroid function testing is a theoretical possibility since Sawin *et al.* (1981) documented an increase in plasma T4 levels in response to ovine TSH injection in *Chrysemys picta*.

Urea

As discussed earlier, the metabolic role of urea is very different to that seen in mammals.

In our hospital, in a cross-section of patients, urea values varied from 0.9–82 mmol/l. Correlation between uric acid levels and urea levels is variable. All of our chelonian patients with uric acid levels in excess of 500 µmol/l were also judged to be hyperuraemic (13–53 mmol/l). However, some animals with very high urea concentrations were normouricaemic. For example, one *Testudo hermanni* with stomatitis and blepharitis was shown to have a urea level of 82 mmol/l but a uric acid level of only 201 µmol/l. This situation may arise where increased protein metabolism and dehydration accompany normal renal function. Water is conserved by resorption from urine retained in the bladder. Since the bladder is also permeable to urea but not urates, hyperuraemia without hyperuricaemia results.

It appears that dehydration in the absence of protein catabolism may not result in hyperuraemia since Christopher *et al.* (1994) found that, in desert tortoises (*Gopherus agassizii*), urea levels did not rise during the dry months of September and October while the animals were actively foraging. In contrast, hibernating animals experienced relative azotaemia.

Gilles-Baillien & Schoffeniels (1965) noted a very marked seasonal variation (Table 7.13) with peak levels of 103 mmol/l at the end of hibernation in April and a trough of only 4 mmol/l in July. Interestingly, although it must be admitted that they do not state the number of animals involved, their urea values for healthy tortoises are, for 11 months of the year, very much higher than reference values used by most commercial laboratories in the United Kingdom.

Christopher *et al.* (1994) found that, in desert tortoises (*Gopherus agassizii*), urea levels were at their highest post-hibernation (mean 16 mmol/l) but fell to a mean of 3 mmol/l in July after rain had fallen.

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As yet it does not appear that hypouraemia is of diagnostic significance.

Uric acid

Uric acid and urates represent the majority of nitrogenous waste in terrestrial reptiles.

Sample lipaemia is a potential cause of artefactually high uric acid values (Mader 1998). Lymphodilution at venepuncture may artificially lower results. Excess dietary protein may promote hyperuricaemia.

In healthy animals levels are likely to be at their highest post-hibernation (especially where underweight animals have relied upon protein catabolism) and lowest in midsummer (Lawrence 1987).

Hyperuricaemia exceeding 1000 µmol/l appears to be a reliable indicator of (intrinsic) renal failure (we have seen cases up to 3830 µmol/l in our hospital) and these animals have a poor prognosis. Normouricaemia does not exclude the possibility of renal pathology.

Moderate dehydration (increases of up to 100 mOsm/l in blood osmotic concentration) did not lead to reduced glomerular filtration rate in desert tortoises (*Gopherus agassizii*) (Dantzler & Schmidt-Nielsen 1996) and might be expected not to cause hyperuricaemia. However, as shown in Table 7.12, fluctuations in excess of 200 mOsm/l may occur in healthy *Testudo hermanni*. At this point it is impossible to say to what extent dehydration alone may lead to a rise in plasma uric acid.

It has been stated that when uric acid levels exceed 1500 µmol/l then urate crystal deposition in the tissues will occur (Zwart 1992). However, uric acid levels may fall again after mineralisation has occurred—we have seen cases with urate deposits although serum levels were well below 1500 at the time of presentation. Normouricaemia does not rule out visceral or articular gout. The threshold level may be affected by other variables and be different for damaged tissues.

We have seen a few individuals with relatively low blood uric acid levels (<100 µmol/l) which subsequently proved to have hepatic pathology.

Vitamin A

Palmer *et al.* (1984) gave values of 10, 20 and 80 IU/g for hepatic vitamin A measured in three apparently healthy, captive *Testudo hermanni*. No information was given as to the diet or supplementation regime.

In animals with iatrogenic hypervitaminosis A characterised by skin sloughing (n = 9), levels were 665–2850 IU/g. In animals which had been parenterally dosed with vitamin A but did not slough (n = 6), levels ranged up to 1020 IU/g.

Raphael *et al.* (1994a) found that in 17 apparently healthy wild-caught pancake tortoises (*Malacochersus tornieri*) plasma retinol levels varied from 0.09–0.77 µg/ml. Males had significantly higher levels (range 0.42–0.77 µg/ml) than females (range 0.21–0.50 µg/ml).

Raphael *et al.* (1995) found that in wild gopher tortoises (*Gopherus polyphemus*) in the United States blood retinol levels were higher in May (0.208 ± 0.9 µg/ml) than October (0.08 ± 0.02 µg/ml).

A juvenile spur-thighed tortoise (*Testudo* spp.) which had received no nutritional supplements was treated at our surgery for pneumonia and blepharitis (confirmed on histopathology). Liver from this animal contained only 1.3 IU/g vitamin A.

Vitamin D (*see also page 71*)

The mean serum 25-hydroxycholecalciferol (25-[OH]D) level in adult *Gopherus agassizii* housed outdoors in Nevada was 8.2 ng/ml (n = 14) with a range from less than 5 ng/ml (n = 3) to 16.5 ng/ml (Ullrey & Bernard 1999).

Apparently healthy juvenile desert tortoises and juvenile African spurred tortoises (*Geochelone sulcata*) housed indoors and fed diets containing about 2000 IU vitamin D_3/kg had serum concentrations of 25[OH]D of less than 5 ng/ml. No measurable changes in serum levels were seen after oral dosing with vitamin D_2/D_3 (Bernard 1995).

Vitamin E

Raphael *et al.* (1994) found that in 17 apparently healthy, wild-caught pancake tortoises (*Malacochersus tornieri*) plasma alpha-tocopherol levels varied from 0.77–5.11 µg/ml and gamma-tocopherol levels from 0.08–1.04 µg/ml. Females had significantly higher levels of alpha-tocopherol (range 2.2–5.1 µg/ml) than males (range 0.78–2.81 µg/ml).

Raphael *et al.* (1995) found that in wild *Gopherus polyphemus* in the United States, blood alpha-tocopherol levels were higher in May (1.46 ± 0.69 µg/ml) than October (0.87 ± 1.05 µg/ml). This difference was significant in females but not in males.

Carnivorous turtles were reported to have much higher levels (Dierenfeld 1989).

ASSESSING HYDRATION STATUS

The ability to quantify fluid deficits is important. The potential for fluid overload may exist when fluids are administered too rapidly by non-oral routes. Patients with renal, respiratory or cardiac dysfunction are particularly at risk of developing oedema although even relatively healthy animals are potentially susceptible. Cardiac disease is probably uncommon but renal disease is certainly not unusual and peripheral oedema is a common sign in these patients even without fluid therapy.

In practice, such an assessment is not easily made. It is frequently stressed in published texts that hydration status be assessed, corrected and maintained, but recommendations as to the details of how this is to be achieved are speculative. A special problem arises in chelonians that use their urinary bladder as a water reservoir. In these animals the first change occurring with the onset of water deprivation is a shift of water from the bladder into other body compartments. Changes in blood parameters and clinical signs of dehydration will certainly be reduced in magnitude (possibly even completely negated) by this shift until bladder water is exhausted. Only at this point does the animal potentially begin to suffer adverse effects of dehydration.

History of weight change

A precise knowledge of weight loss is an invaluable aid in the quantification of dehydration, particularly in the acute case. It will never be an entirely accurate measure, since variables such as loss of gut content, fat reserves or body protein, oviposition,

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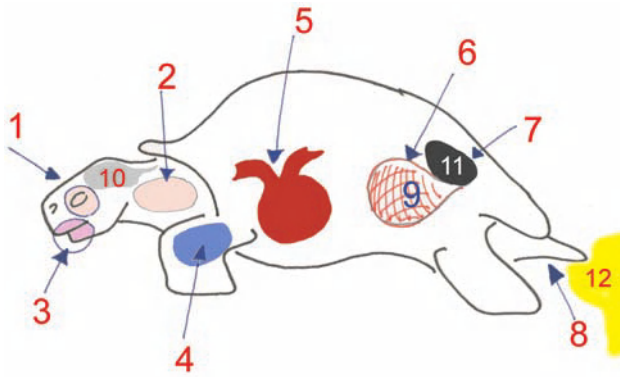

Fig. 7.24 Dehydration in a uricotelic terrestrial chelonian.
(1) Sunken eyes
(2) Decreased skin elasticity
(3) Dry, tacky mucous membranes
(4) Muscular weakness
(5) Altered plasma biochemistry
(6) Precipitation if urate within the bladder is increased
(7) Loss of glomerular filtration
(8) Decreased urine output
(9) Exhaustion of bladder fluid reserve
(10) Gouty deposits within the brain
(11) Gouty deposits within the kidneys and other visceral organs, such the heart, and joints
(12) Alterations in urine specific gravity (SG) (increased) and pH (decreased)

urination and tissue oedema must be taken into account. Many chelonian owners/keepers are highly motivated and can readily be persuaded to weigh their pets accurately every couple of weeks.

Clinical signs

Both Klingenberg (1996) and Bennett (1998c) suggest that, in reptiles, hydration status can be assessed in much the same manner as in mammals. They state that mild (5%–8%) dehydration is characterised by loss of skin elasticity and skin 'wrinkling'. With further deterioration the eyes appear sunken and the mucous membranes become dry and tacky. Some caution must be exercised since healthy reptiles have drier mucous membranes than mammals. Sunken eyes and loss of skin elasticity may also result from cachexia (Fig. 7.24).

Haematocrit

An abnormally high PCV is consistent with dehydration. However, variables such as species, sex (males have higher PCV in some species) and season must be taken into consideration. Anaemia is probably common in chronically-ill reptiles and may mask an elevation in PCV.

Blood biochemistry

As with PCV, abnormally raised blood albumin is consistent with dehydration, but hypoproteinaemia is also common in sick chelonians and may mask the effects of dehydration. An added complication is the phenomenon of elevated breeding-season albumin in females of many species.

Klingenberg (1996) states that dehydration should be considered as a possible cause of elevation in blood uric acid. However, the work of Dantzler & Schmidt-Nielson (1996) suggests that glomerular filtration rates and hence blood uric acid levels in terrestrial chelonians may actually be quite resistant to water deprivation.

Uric acid and urea

Our experience suggests that elevated blood uric acid levels (>1000 µmol/l) are likely to indicate renal insufficiency. There may or may not be concurrent fluid/electrolyte imbalance.

Blood urea has been widely discounted as a useful indicator in reptilian biochemistry. However, this may not be true for groups, such as terrestrial chelonians, where urine is retained in the bladder as a fluid reserve against periods of water deprivation. Urea readily crosses biological membranes and may equilibrate between bladder urine and blood—in contrast to uric acid which is precipitated in the bladder as insoluble urates.

At Holly House, hyperuricaemic patients are almost invariably also hyperuraemic. A further subset of cases, however, is normouricaemic but hyperuraemic. Many of this group seem likely, from history and physical examination, to be dehydrated. This theory is supported by the observation that healthy hibernating *Testudo hermanni* and *Gopherus agassizii* become hyperuraemic towards the end of hibernation (Gilles-Baillien & Schoffeniels 1965; Christopher *et al.* 1994). The work of Christopher *et al.* (1994) suggests, however, that dehydration in the absence of protein catabolism will not induce azotaemia.

A normal blood uric acid level in conjunction with elevated urea may indicate dehydration and protein catabolism with normal renal function. A normal urea level in a non-catabolic animal does not exclude the possibility of dehydration.

Urine specific gravity (SG)

Innis (1997b) found that the specific gravity of urine from a variety of terrestrial herbivorous species maintained in captivity and apparently healthy varied from 1.003–1.012. In two animals emerging from hibernation (enforced dehydration) urine specific gravity was 1.008 and 1.012.

Christopher *et al.* (1994) found that at peak rainfall in July desert tortoises (*Gopherus agassizii*) produced urine with specific gravity of 1.007 ± 0.002. At other times of the year urine was significantly more concentrated, rising to a maximum of 1.017 ± 0.012 at emergence from hibernation.

It would seem that urine SG is a potentially useful indicator of hydration status in terrestrial chelonians. In contrast, Prange & Greenwald (1980) showed that dehydration had little effect on urine electrolyte concentrations in marine turtles, since the salt gland appears to play the dominant role in homeostasis.

Tear gland secretion

Prange & Greenwald (1980) demonstrated that the tear gland secretion of green turtles (*Chelonia mydas*) varies in composition with hydration status (Table 7.16).

Terrestrial reptiles may also produce salt-rich secretions from their noses which may vary in composition with hydration status

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Table 7.16 Composition of tear gland secretion in green turtles (*Chelonia mydas*) under different conditions of hydration (Prange & Greenwald 1980).

	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)
Normal	388	26	401
Dehydrated (10–20 days out of water)	834	28	1020
Rehydrated in fresh water	215	38	222

(Schmidt-Nielson *et al.* 1963). However, we are unaware of any studies relating directly to terrestrial chelonians.

Blood osmolality and its implications for fluid therapy

There has been some debate in the past about the ideal osmolality of fluids for intravenous/intracoelomic infusion in chelonians. The data presented in Table 7.17 below and in Tables 7.14 and 7.13 above suggest that at least terrestrial and marine chelonians can tolerate a wide range of plasma osmolalities without adverse effect. The data for *Testudo hermanni* and *Caretta caretta* is very much more complete than for the other species listed, and demonstrate the pronounced seasonal variation which probably occurs in most temperate species.

It may, therefore, be appropriate to take advantage of electrolyte solutions isotonic to mammalian blood (which generally has total osmolality in the region 280–310 mOsm/l). The osmolality of blood in a given patient can be approximated using the equation:

$$\text{Osmolality} = 2\,[\text{Na} + \text{K}] + \text{glucose} + \text{urea}\ \text{(all measured in mmol/l)}$$

Michell *et al.* (1989) give pre-treatment blood osmotic concentration for some representative patients at Holly House, calculated using this equation (Table 7.18).

The difference between this data and that for healthy *T. hermanni* is not straightforward to interpret since our patients include animals which did not hibernate and those which did seem likely to have emerged earlier than those of Gilles-Baillien & Schoffeniels (1965). It appears, however, that the tendency in this small series is for our chronically sick animals to be isotonic or hypotonic to non-hibernating, healthy chelonians. Even the animal with stomatitis, which might be expected to experience difficulty in drinking, had no evidence of hypertonicity.

If fluids are to be administered it seems reasonable that they should include a significant amount of sodium. Hypernatraemia seems to be uncommon in our patients (our maximum of 163 mmol/l for a patient at Holly House is lower than the maximum recorded in healthy animals post-hibernation by Gilles-Baillien & Schoffeniels).

COAGULATION PARAMETERS

The only information available on coagulation parameters stems from the work of Seccareccia *et al.* (1997) who measured prothrombin time (PT), partial thromboplastin time (PTT) and plasma fibrinogen in healthy captive *Geochelone radiata* and *Geochelone pardalis* (Table 7.19).

Virtually nothing is known of reptilian coagulation pathways. The authors were unable to distinguish between actual or artefactually high PT and PTT (as compared with canine values).

BONE MARROW BIOPSY

A technique for bone marrow biopsy is described in Frye (1991a). We do not ourselves have experience of using it. Histological examination is useful for assessing overall marrow cellularity.

Marrow may be retrieved from the shell osteoderms by boring through the outer layer only and withdrawing a sample with a suitable biopsy needle (e.g. Vim-Silverman). Garner *et al.* (1996) found that the pelvis, proximal humerus, proximal femur and 'the thickened cranial and caudal regions of the carapace and plastron' were suitable sites for bone marrow collection. They particularly recommended the gular plates of the plastron, which have a thick medulla and are readily accessible for core or wedge biopsy. The shell defect should be repaired with resin.

Table 7.17 Blood osmotic concentration in various chelonian species.

Species	Blood osmotic concentration (mOsm/l)	Circumstances	Reference
Testudo hermanni	258–449	normal seasonal variation	Gilles-Baillien & Schoffeniels (1965)
Gopherus agassizii	291–334	wild animals, summer	Minnich (1976)
Caretta caretta	465	sea water	Schoffeniels & Tercafs (1965)
	300–343	normal seasonal variation in wild adults	Lutz & Dunbar-Cooper (1987)
	224	after three years in fresh water	Schoffeniels & Tercafs (1965)
Chelonia mydas	270	sea water	Holmes & McBean (1964)
juveniles	207	after two months in fresh water	Holmes & McBean (1964)
Chelydra serpentina	315	fresh water	Semple *et al.* (1969)
Trionyx spiniferus	280	fresh water	Dunson & Weymouth (1965)
Trachemys scripta	260	fresh water	Hutton & Goodnight (1957)

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Table 7.18 Blood osmolality in representative clinical cases.

Species	Month	Condition	Approx. blood osmolality
Furculachelys whitei	March	upper respiratory tract infection	295
Testudo graeca	April	stomatitis	291
Testudo spp.	March	ovarian stasis?	291
Testudo hermanni	June	chronic 'ill-thrift'	227
Testudo spp.	March	rhinitis	296
Testudo graeca	March	chronic 'ill-thrift'	300
Geochelone pardalis	April	viraemia?	277

Table 7.19 Coagulation parameters in healthy chelonians.

Species	PT (sec)	PTT (sec)	fibrinogen (mg/dl)
Geochelone radiata (n = 6)	14.8–33.8	5/6 animals >100	<50*
Geochelone pardalis (n = 3)	13.4–42.6	21–36	240–300

* Three animals (not included) were anaesthetised with intramuscular propofol plus hyaluronidase and had fibrinogen values 200, 800, 800 mg/dl. Those included in the table received propofol intravenously.

Table 7.20 Cellular composition of bone marrow from desert tortoises (*Gopherus agassizii*) (n=16) as evaluated by cytological examination after saline-soak extraction (Garner *et al.* 1996).

Cell type	% of cells
Heterophils	65
Myeloblasts	21
Eosinophils	2
Basophils	0.5
Monocytes/monoblasts	2
Azurophilic monocytes	<1
Lymphocytes	2
Undifferentiated blast cells	1
Erythrocyte precursors	6

Unlike mammals, birds and other reptiles, chelonians lack gelatinous marrow. Marrow cellularity is low and the network of bony trabeculae hinders access. Garner *et al.* prepared specimens for cytological examination using a saline-soak technique to extract cells from the marrow samples (Table 7.20). Harvested material was cut into 2 mm-thick sections, placed in an EDTA-containing evacuated tube and incubated at 4°C for 18–24 hours in 2 ml phosphate-buffered saline. After agitation for 30 minutes, 200 µl samples of solution were centrifuged and mounted onto slides for staining.

This method allowed more rapid processing and easier differentiation of cell types than conventional decalcified histological sections and may be the technique of choice for bone-marrow evaluation in chelonians in both live animals and at post mortem.

Garner *et al.* (1996) described myeloblasts as having 'a moderate amount of agranular, lightly basophilic cytoplasm and a large, central to slightly eccentric ovoid, vesicular nucleus with a large nucleolus'. Developing cells acquired 'variable numbers of slightly elongated pink granules'. Immature eosinophils were not distinguished from 'undetermined blast cells'. Monoblasts were difficult to differentiate from myeloblasts unless special stains were used. Cells of the erythrocyte series had variably-sized, central, round nuclei and a variable amount of pale blue cytoplasm. The origin(s) of thrombocytes remains obscure. Lymphocytes may stem from the thymus and spleen.

In this small series there was a tendency for heterophilic animals to have reduced numbers of marrow heterophils and for heteropaenic animals to have hyperplastic marrow.

CYTOLOGY

Cytology is a cheap tool which may allow a provisional diagnosis to be arrived at quickly. This gives a rational basis for immediate treatment measures and allows prioritisation of subsequent investigations such as histopathology, mycobacterial culture, fungal culture, biochemistry, etc. There is one particular advantage of cytology over histopathology in chelonians: heterophil cytoplasmic granules may be difficult to distinguish from those of eosinophils after processing for histopathological examination.

The staining technique(s) employed will depend upon circumstances. Rapid stains are often adequate for cellular morphology and will often stain pathogens. Furthermore, specific stains for infectious agents, such as Gram's (bacteria), methylene blue (fungi) or Ziehl-Neelsen (mycobacteria) may be necessary.

Urate crystals are an important finding. In urine or other fluids they are needle-shaped, but in tissue samples they may appear round with an X pattern—they may be easier to see with cross-polarised light microscopy (Frye 1991a). It is important to be aware that methanol fixative does not dissolve away urates whereas aqueous solutions such as formol saline will do so.

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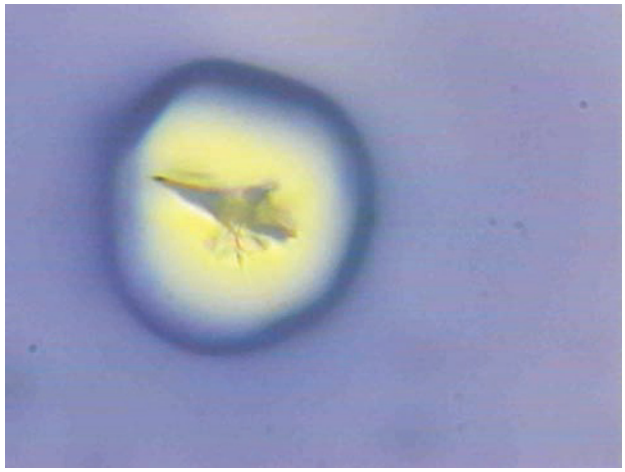

Fig. 7.25 A grain of corn flour (corn starch) from **glove powder** in a cytological preparation. Rapi-diff staining; approximately × 1000 magnification.

These are frequently present when clinicians use powdered gloves and should not be mistaken for urate crystals. The irregular rounded outline and central 'star' are characteristic.

Beware starch grains from glove powder, which appear as irregular crystalline spheres (Fig. 7.25).

The *murexide test* is a valuable tool for the confirmation of urates and their differentiation from calcium salts (Speer 1997). A drop of nitric acid is mixed with the crystals on the slide, which is then slowly dried over a flame. When one drop of ammonia is added, a red-purple colour indicates the presence of urates. These reagents are routinely employed by veterinary laboratories in the identification of urate uroliths in other species and are widely available. However, it seems that a significant amount of urate is required to elicit a detectable colour change. The author has not had success in using this technique to prove the identity of suspected urates within fine-needle aspirates.

If cytology preparations are to be sent away for examination by an external laboratory they should be fixed prior to dispatch. In oral impression smears, for example, all cellular detail will have been obliterated by bacterial overgrowth within 24–48 hours unless fixed.

Skin and shell

Exfoliating cells and organisms can be collected with acetate-strip impressions. These can be directly immersed in stain (rapid stains are usually sufficient) before rinsing, applying to a slide and viewing under a cover slip. Alternatively, material can be collected by scraping or direct impression smear from the skin or exfoliated matter (e.g. from separated scutes). Information thus gained about the relative abundance of bacteria, yeasts, and fungal elements provides complementary information to qualitative information from culture.

Masters *et al.* (1995) described the first isolation of *Dermatophilus cheloniae* from skin lesions in captive chelonians. This organism appears as distinctive branching chains of Gram positive cocci up to five units wide. Motile zoospores might also be seen in fresh preparations. Rebell *et al.* (1975) found intranuclear inclusions in superficial keratinocytes from turtles affected with 'grey-patch disease', which may be caused by a herpesvirus. Pox virus lesions in a Hermann's tortoise were characterised cytologically by eosinophilic cytoplasmic inclusions (Orós *et al.* 1998). Jacobson *et al.* (1991a) reported ballooning degeneration and eosinophilic intranuclear inclusions in the skin lesions of green turtle fibropapillomatosis.

Cutaneous squamous cell carcinomas have been reported in several chelonians (Cowan 1968).

Oral cavity

The lining of the oral cavity may be swabbed with a cotton bud and gently rolled onto a slide. Alternatively, a slide may be pressed directly onto the tongue of a cooperative animal. This can be examined directly for parasites or air-dried for staining. In normal animals both anuclear and nucleated epithelial cells are present, and are accompanied by small numbers of cocci. Some of the nucleated cells, presumably from more basal layers, will exhibit one or two small, well-defined nucleoli.

In stomatitis cases the proportion of nucleated cells increases and nucleoli are present in a greater proportion. Other findings of general interest include heterophils (indicative of stomatitis as in Fig. 7.26), inclusion bodies (viral stomatitis as in Fig. 7.22), fungal elements, yeasts, bacteria (often vast numbers of Gram negatives in stomatitis cases) and trematode eggs.

Most published matter refers to eosinophilic intranuclear inclusion bodies in epithelial cells of tortoises affected by herpesvirus (e.g. Marschang *et al.* 1997a). Using Rapi-Diff® stain, however, such cases at our surgery characteristically exhibit large intranuclear inclusion bodies (occupying 25%–50% of the nucleus) that appear pale grey with a darker margin. Jacobson (1999: personal communication) reports that the appearance of herpetic inclusion bodies under light microscopy depends very much upon the stain used and the particular virus involved.

Respiratory and gastrointestinal tract disease has also been associated with an irido-like virus (Marschang *et al.* 1998b). Iridovirus infection is characterised histopathologically by basophilic intracytoplasmic inclusion bodies. Apparent nucleoli are also commonly seen in the nuclei of basal epithelial cells from animals with stomatitis (Fig. 7.26). Confirmation of the identity

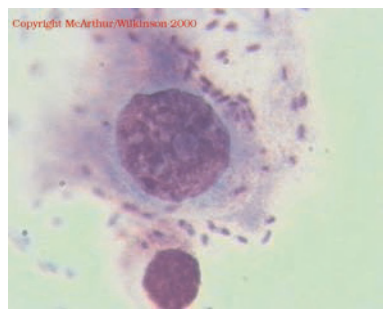

Fig. 7.26 Cytological preparation. A tongue impression smear from a spur-thighed tortoise (Testudo sp.) with severe stomatitis, prior to antibiotic therapy. Rapi-Diff staining; approximately × 1000 magnification.

The upper cell is an epithelial cell with a large nucleolus. Below it lies a degenerate heterophil (with the remnants of orange cytoplasmic granules). Numerous bacteria are present.

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of inclusions will ultimately depend upon immunocytochemical techniques. Cellular details, such as inclusion bodies, are likely to be much easier to see in animals that have been pre-treated with a broad-spectrum antibiotic, otherwise profuse bacterial growth may lead to degeneration of any cells present.

Respiratory system

Tracheal or bronchial washes can be obtained relatively easily under sedation by inserting a measured cat urinary catheter through the glottis and flushing with a few millilitres of sterile saline before aspirating. It is important to be aware of the fact that respiratory lesions may well be unilateral and that since the tracheal bifurcation lies close to the glottis, a sample from the wrong side may completely lack relevant changes.

Inclusion bodies and giant cells have been seen in epithelial cells from the respiratory tracts of tortoises and sea turtles with suspected iridovirus and herpesvirus infections (Westhouse *et al.* 1996; Pettan-Brewer *et al.* 1996). Bacteria are present as primary or secondary pathogens in many animals with respiratory disease and a variety of fungi have been isolated from mycotic pneumonias (Jacobson 1997). *Aspergillus*, usually characterised by septate branching hyphae, may be the commonest of these. Pulmonary neoplasms are rare (Jacobson 1997).

Coelomic fluid

It is difficult to retrieve fluid from the coelomic cavity of healthy animals (beware inadvertent cystocentesis). Such samples might contain mesothelial cells. Transudates and modified transudates have low cellularity and may require slow-speed centrifugation (or simply allow the sample to stand for a few hours) to concentrate the cells. Exudates are highly-cellular fluids characterised by the presence of heterophils and/or macrophages and inciting agents such as bacteria, fungi, *Hexamita* or needle-shaped urates. Egg coelomitis is recognised by the presence of deeply-basophilic, variably-sized yolk droplets (Dorrestein 1997).

Soft-tissue masses

Samples of soft-tissue masses for cytology can be collected by fine-needle aspiration (FNA) or prepared as impression smears from the cut surface of excised samples.

Acute inflammation is characterised by the presence of heterophils. This is succeeded (at a rate dependent upon variables such as temperature, nutrition and the nature of the stimulus) by chronic inflammatory change characterised by heterophils plus macrophages (which predominate in mycobacterial, fungal or foreign-body reactions) and sometimes by giant cells (Montali 1988).

Infectious agents include bacteria (cocci, bacilli and acid-fasts), fungi and yeasts. Figure 7.27 illustrates hyphae in a needle-aspirate of a lung abscess from which *Paecilomyces* was cultured.

Mineral crystals (particularly urates in gout and hydroxyapatite in pseudogout) are important findings. Pseudogout in subcutaneous tissue is characterised by finely-granular eosinophilic debris, macrophages and giant cells (Frye & Dutra 1976).

A few cases of neoplasia potentially amenable to cytological diagnosis have been reported in chelonians (Cooper *et al.* 1983).

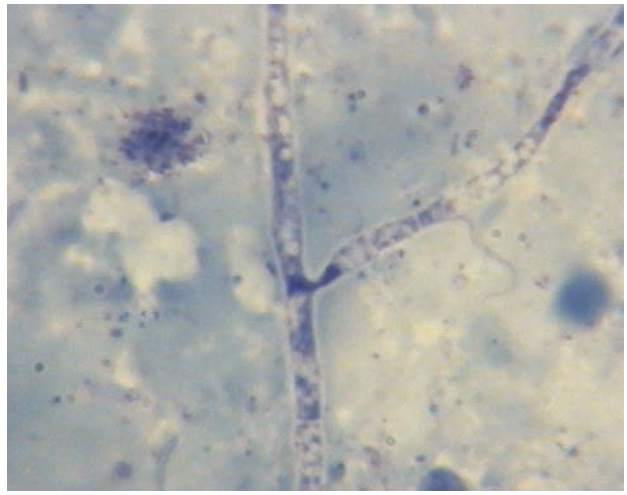

Fig. 7.27 Fungal hyphae in a fine-needle aspirate from a mycotic lung abscess in a leopard tortoise (*Geochelone pardalis*). Rapi-Diff staining; approximately × 1000 magnification.

Multiple bacteria types are also present. Mixed infections are common in chelonians.

Organs affected by neoplasia to date include the skin, thyroid, parathyroid, lungs, stomach, gallbladder, testes and kidneys (Done 1996a).

In contrast to mammals, truly eosinophilic tissue responses are not recognised in reptilians (Montali 1988) although clinical evidence of apparent allergic reactions has been observed (Frye 1999).

Joint fluid

Swollen joints are not uncommon as a presenting sign. Gout (fluid grossly whitish and characterised microscopically by urate crystals plus inflammatory cells) and septic arthritis (characterised by degenerate heterophils, macrophages and sometimes visible micro-organisms) are the primary differentials, although a systemic lupus erythematosus-like disease has been described in iguanas (Frye 1991a). In pseudogout, hydroxyapatite crystals are deposited in joints. Unlike the urate crystals of true gout, these crystals are non-birefringent under polarised light (Frye & Dutra 1976).

Cerebrospinal fluid (CSF)

Owens & Rutz (1980) describe CSF sampling for cytology in marine turtles with neurological signs. With the neck flexed ventrally, a 5–8 cm needle is directed ventrally and slightly cranially into the neck in the midline just caudal to the supraoccipital protrusion. Two to three ml of fluid may be withdrawn from a 20–40 kg turtle. Cytology provides the possibility of an antemortem diagnosis of viral, bacterial, mycobacterial or fungal meningitis—which have all been demonstrated histopathologically in reptiles (e.g. in cases of hind limb paralysis) (Wenker *et al.* 1997).

Table 7.21 gives a guide to the interpretation of cytological findings from different sites.

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Table 7.21 Guide to interpretation of cytology findings.

Site and Preparation	Normal findings	Disease	Pathological cytology
Oral—impression preparation	mixture of nucleated and anuclear squamous epithelial cells (Fig. 7.28); few bacteria, mostly cocci	herpesvirus stomatitis iridovirus stomatitis	heterophils (Fig. 7.26); increased numbers of nucleated epithelial cells; vast numbers of bacilli; sometimes yeasts; sometimes eosinophilic intranuclear inclusion bodies as herpesvirus but basophilic intracytoplasmic inclusions
Skin and shell—impression preparation; fine-needle aspirate	low numbers of bacteria	trauma; burns; hypervitaminosis A; septicaemic cutaneous ulcerative disease (SCUD) in freshwater species	heterophils; vast numbers of bacilli (Fig. 7.29); sometimes yeasts
		mycoses	heterophils; macrophages; fungal hyphae
		Pox virus	eosinophilic intracytoplasmic inclusion bodies
		squamous cell carcinoma	criteria of malignancy
Lower respiratory tract—bronchial lavage, sample both sides	no infectious agents; occasional epithelial cells	herpesvirus	heterophils; bacteria; eosinophilic inclusions
		iridovirus	heterophils; bacteria; basophilic inclusions
		neoplasia	(two reports) cytology not performed
		bacterial pneumonia	heterophils; bacteria (usually bacilli)
		fungal pneumonia	heterophils; macrophages; hyphae (*Aspergillus* has branching, septate hyphae)
Coelomic fluid—fine needle aspirate	difficult to retrieve any fluid (beware bladder puncture)	coelomitis	heterophils; sometimes macrophages plus inciting agents such as bacteria, fungi, egg yolk (basophilic globules), *Hexamita* flagellates or urate crystals
Soft-tissue masses—fine needle aspirate	absence of inflammation and infectious agents	infections	heterophils; macrophages plus agent(s) (Fig. 7.27) acid-fast staining for mycobacteria is important
		gout	urate crystals (X-patterned, distinguish from glove powder!); heterophils; macrophages
		pseudogout (rare)	finely-granular eosinophilic debris; macrophages; giant cells
		neoplasia	criteria of malignancy
Joint fluid—fine needle aspirate	difficult to retrieve; minimal cells present.	septic arthritis	heterophils; bacteria (usually bacilli)
		gout	heterophils; macrophages; urate crystals (Fig. 7.30)
		pseudogout (rare)	hydroxyapatite crystals are non-birefringent under polarised light—unlike urates
Cerebrospinal fluid	almost acellular	meningitis	viral, bacterial, mycobacterial, or fungal aetiologies have been reported in reptiles

PARASITOLOGY AND FAECAL EXAMINATION

Ectoparasites

External parasites are uncommon in animals that have not recently been captured from the wild. Cloacal mites of the genus *Cloacaridae* and *Sarcophaga* fly larvae are described below with endoparasites. Ticks are the commonest ectoparasites and are potentially important vectors of disease.

Brotóns Campillo & Frye (2002) describe a case of apparent acariasis in an African spurred tortoise (*Geochelone sulcata*). Numerous mites of all life-cycle stages were present in underrunning lesions of the carapace scutes. The identification of the mites has yet to be determined. In the United Kingdom, mites observed on a Hermann's tortoise *Testudo hermanni* at post mortem (M. Barrows: personal communication) appeared similar to *Glycyphagus*—a forage mite (R. Wilkinson: unpublished).

Endoparasites

Endoparasites are extremely common both in long-term captive chelonians, often despite anthelminthic treatment, and in wild animals. The incidence of parasitism may vary widely from

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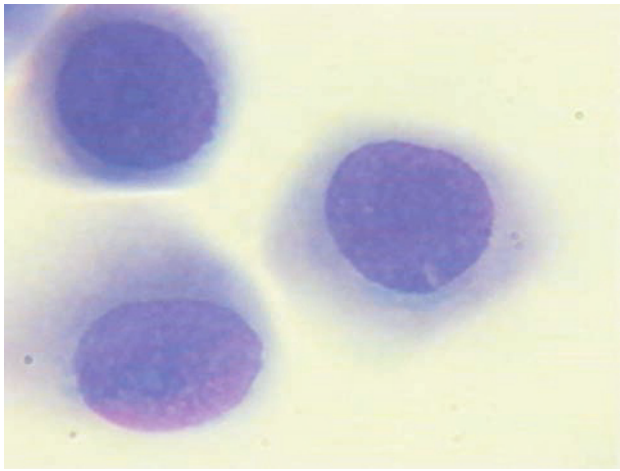

Fig. 7.28 Cytological preparation. A **tongue impression smear** from a healthy (*Testudo* sp.). Rapi-Diff staining; approximately × 1000 magnification.

Bacteria are normally sparse or absent in preparations from the mouths of healthy chelonians. Prominent nucleoli are less often present in epithelial cells since the basal layers do not normally exfoliate.

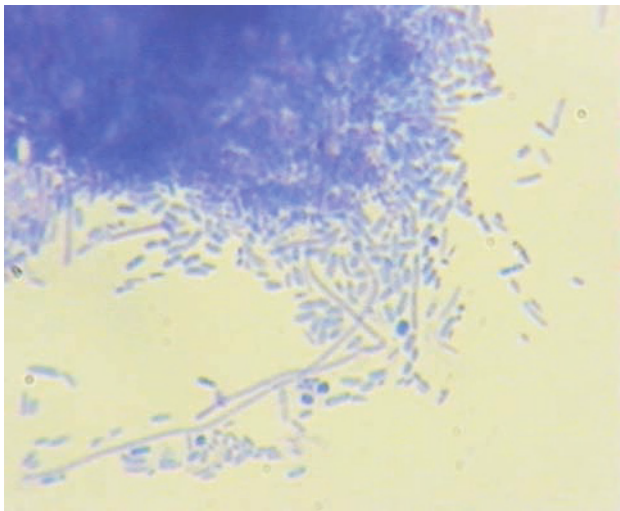

Fig. 7.29 **Rod-shaped bacteria** in a preparation from a skin lesion on a soft-shell turtle (*Trionyx* sp.) Rapi-Diff staining; approximately × 1000 magnification.

Gram-negative rods belonging to the Enterobacteriaceae are opportunistic pathogens and are responsible for the vast majority of bacterial disorders in chelonians.

species to species. Martin (1972) found that wild-caught animals of some species had no parasites whilst every individual of other species from the same area were infested. In general, carnivorous aquatic species were more commonly parasitised and carried a wider variety of metazoan parasite types—often nematodes, trematodes, acanthocephalans and leeches. Terrestrial chelonians were typically affected by nematodes alone.

Faecal examination

In rare cases, helminth eggs or larvae can be seen in oral mucus, tracheal washes or urine. Protozoa are occasionally evident in blood, coelomic fluid or in other tissues such as liver. For most metazoan parasites however, faecal analysis is the most important

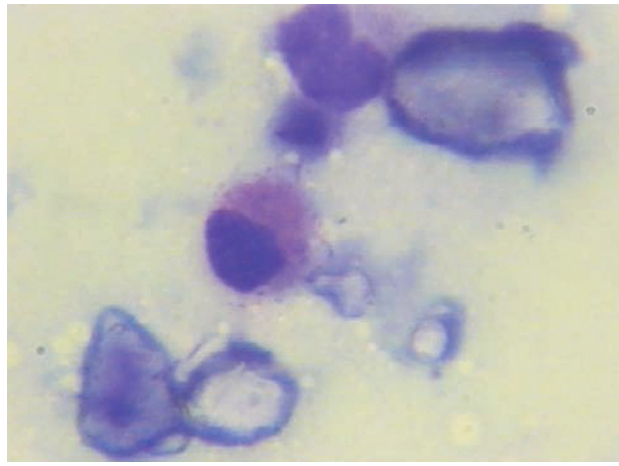

Fig. 7.30 Amorphous **urate crystals** and a heterophil (centre) in a joint fluid aspirate from a spur-thighed tortoise (*Testudo* sp.) with articular **gout**. Rapi-Diff staining; approximately × 1000 magnification.

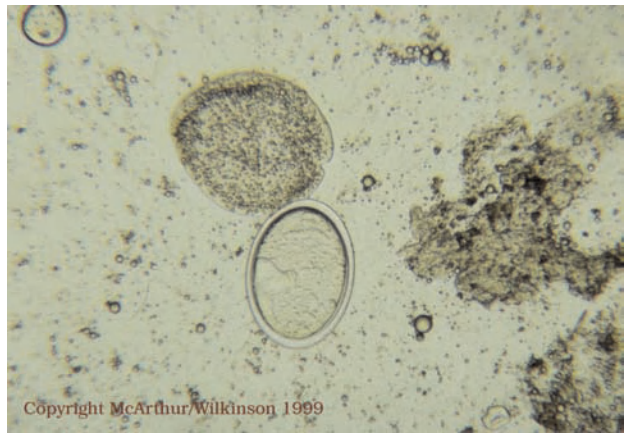

Fig. 7.31 An **ascarid egg** *Angusticaecum* sp. (below) and a **ciliate cyst** (above) in a faecal wet smear from a healthy *Testudo graeca graeca*. Unstained; approximately × 100 magnification.

Ascarid eggs are round/ovoid and thick walled with scalloped external margins .

procedure. Fresh faecal samples are preferable, since protozoa will be active and eggs are less likely to have hatched. Flagellates, in particular, will be hard to detect after a couple of days. Refrigerated samples may be representative for up to two days. Faecal samples may be formalinised to preserve protozoal trophozoites if examination is to be delayed. Uncooperative patients may be persuaded to part with a sample by flushing the cloaca, coprodeum or caudal colon with a few millilitres of water. Some will defecate when bathed in warm water.

Wet smears

Wet smears give a rapid indication of the organisms present (Figs 7.31–7.35). A small amount of faeces is liquefied in a few drops of saline and examined (usually at × 10 magnification) under a cover slip. When it is necessary to immobilise active organisms for identification, a couple of drops of 10% formol-saline, Lugol's iodine (also stains protozoa and their cysts when used at double strength), mercurochrome or merthiolate will suffice. Phase-contrast light microscopy is a very good tool to identify, and most often also classify, the protozoans visible in a wet mount.

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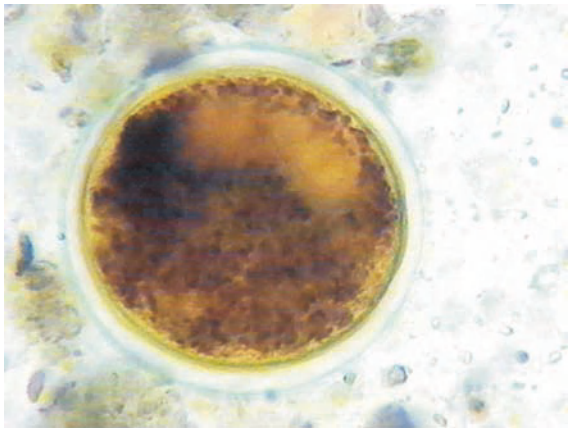

Fig. 7.32 An **ascarid egg** *Angusticaecum* sp. in a faecal wet smear from a healthy *Geochelone sulcata*. Stained with Lugol's iodine; approximately × 400 magnification.

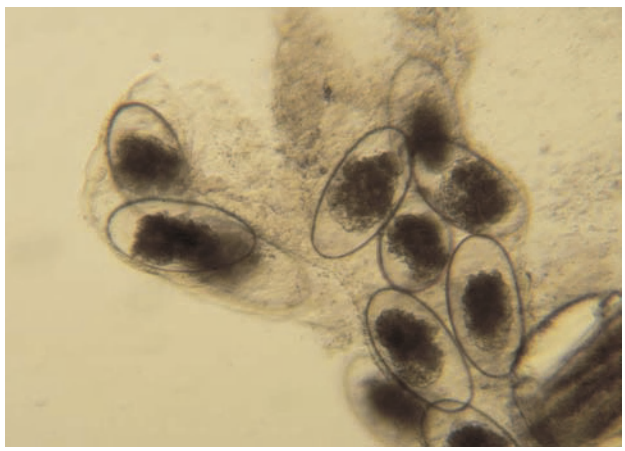

Fig. 7.33 **Oxyurid eggs** in a faecal wet smear from a Mediterranean tortoise (*Testudo sp.*). Unstained; approximately × 100 magnification.

Oxyurid eggs are common in the faeces of captive *Testudo* tortoises. They are elongated, ovoid and may be asymmetrical (D-shaped). Their walls are relatively thin and smooth on the outer margin.

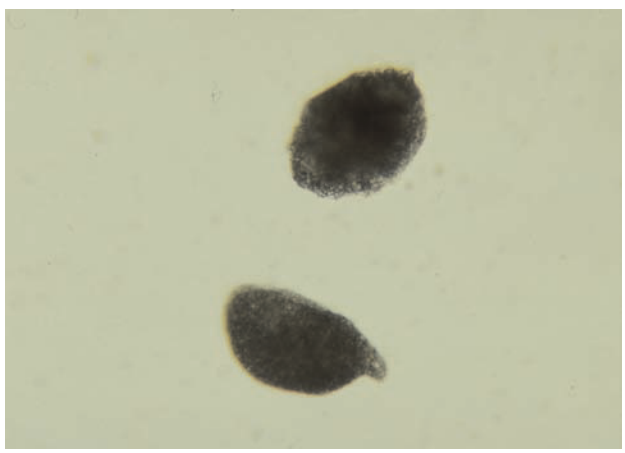

Fig. 7.34 Two motile ***Balantidium*** trophozoites in a faecal wet smear from a healthy *Testudo graeca*. Unstained; approximately × 100 magnification.

These small ciliates are common commensals.

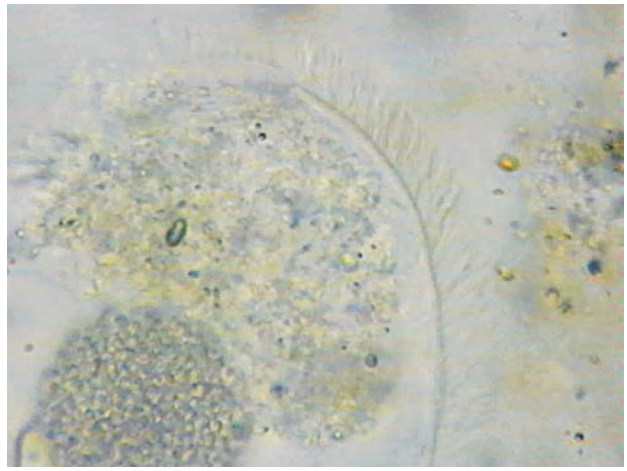

Fig. 7.35 Part of ***Nyctotherus***: trophozoites in a faecal wet smear from a healthy *Geochelone sulcata*. Unstained; approximately × 100 magnification.

This, the other common ciliate of terrestrial chelonians, is very large with long cilia.

Flotation

Flotation concentrates parasite eggs (ascarids, oxyurids) and cysts (including coccidian oocysts). Sucrose and zinc-sulphate solutions are the most widely-used media. Spirurid, cestode and trematode eggs may not float in sucrose. Zinc sulphate is better in this respect, but slides should be examined within 20 minutes to avoid crystallisation.

If quantitative data is required, a weighed amount of faeces is suspended in a measured volume of water. The coarse matter is filtered out and the filtrate is centrifuged. The supernatant is then discarded and the sediment re-suspended in a volume of saturated salt or sugar solution equal to that of the discarded supernatant. The counting chamber of a McMaster slide is filled with the sample and left to settle with a cover slip on. The faecal worm egg count can be calculated assuming the volume of the counting chamber is known.

Stained, air-dried smear

Smears can be stained with rapid stain, Gram's, methylene blue or an acid-fast stain, according to which organism is suspected. Klingenberg (2000) recommends carbolfuschin with brilliant green counterstain for cryptosporidium which then appears dark red against a green background. Although of limited application this method is useful for detecting gastrointestinal yeast overgrowth.

Sedimentation

Centrifuge and examine the sediment for protozoans, their cysts and trematode eggs, which may not be detected by flotation methods.

Reptile faeces often contain structured elements which can easily be confused with potential pathogens. Herbivore faeces may include undigested pollen, fungal spores, seeds, worm-like plant elements, cell walls (Figs 7.36–7.37) and the bodies of arthropods found on forage. Carnivores may pass endo- or ectoparasites (and their eggs) of their prey species. Ova of the earthworm parasite *Monocystis* are often seen in faeces of worm-eating chelonians (Innis 2002: personal communication). They

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Fig. 7.36 Plant matter in a faecal wet smear from a healthy *Testudo*. Unstained; approximately × 100 magnification.

Faecal preparations contain a wealth of potential pitfalls for the unwary pathologist. A myriad of different plant elements, plant parasites, prey parasites and eggs of prey parasites are passed out undigested.

Fig. 7.37 Pollen in a faecal wet smear from a healthy box turtle *Terrapene*. Unstained; approximately × 100 magnification.

resemble *Trichuris* or *Capillaria* ova but are smaller and are often found in 'packets' containing several ova. *Monocystis* ova have often been mistaken for true parasites of chelonians. They appear to be non-pathogenic.

Identification of faecal endoparasites

There are no comprehensive illustrated guides to the identification of helminth eggs. Information can be gleaned from a variety of sources such as the papers by Barnard (1986a–d) or Frye (1991a), but many well-researched papers still refer to unidentified helminth eggs. Frank & Reichel (1977) suggest that, when difficulty is experienced in distinguishing cestode from trematode eggs, the sample should incubated at room temperature for 14 days and reinspected for the development of miracidia (trematodes) or oncospheres (cestodes).

Table 7.22 below summarises the characteristics by which organisms may be recognised, and their clinical significance.

URINALYSIS

Urinalysis is an under-used and valuable diagnostic. Voided urine can often be collected from tortoises without undue difficulty. In fact, in small animals urine is often much easier to obtain than blood. Cystocentesis is possible through the inguinal fossa cranial to the hind limb and is facilitated by ultrasonographic imaging. However, animals will often urinate when agitated under restraint e.g. for blood sampling or ultrasound. Digital stimulation of the cloaca and bathing may also trigger reflex urination.

Urine solids

Whilst marine turtles produce homogeneously liquid urine (Prange & Greenwald 1980), terrestrial chelonian urine usually consists of a fluid fraction and a solid 'pellet'. Innis (1997b) observed crystals which were generally amorphous but occasionally spherical or diamond-shaped on microscopy.

The nature of the nitrogenous excretion product may vary with water availability. Minnich (1972) demonstrated that some 88% of the pellet dry weight was made up of urate in the terrestrial species investigated. Since urate salts are of low solubility and herbivorous reptile urine is generally alkaline, over 90% of this urate is present as urates rather than as uric acid. Sodium, potassium and ammonium urate predominate. The amount of potassium present is much higher in herbivorous species.

For the desert tortoise (*Gopherus agassizii*) Minnich (1972) estimated the electrolyte content (mEq/kg dry weight) of the urinary solids as shown in Table 7.23.

No data are available for carnivorous or omnivorous chelonians, but in snakes up to 74% of urate is in the form of monoammonium urate.

Cystic calculi

Bladder stones are not uncommon in captive chelonians that eat diets of inappropriate mineral content. Mader & Ling (1999) reported on 100 cases of captive desert tortoises (*Gopherus agassizii*), of which all samples analysed were found to contain urates. In addition to the expected urates, we (RW & SM) have also seen apparent ammonium oxalate (in Mediterranean *Testudo* tortoises) and cholesterol-containing (in one *Geochelone sulcata*) calculi. Nephrolithiasis has been reported in hypervitaminosis D (Ullrey & Bernard 1999). A cystic calculus has also been recorded in a wild Western spiny soft-shell turtle, *Apalone* (*Trionyx*) *spiniferus* (McKown 1998). The chemical components in this case were apatite (basic calcium phosphate), struvite (magnesium ammonium phosphate hexahydrate) and unidentified crystals.

Specific gravity (SG)

Anatomical and physiological considerations complicate interpretation of the composition of the fluid part of the urine. Chelonian kidneys invariably produce hypo- or isosmotic urine. This urine is further modified before voiding by water and electrolyte exchange across the bladder wall (Dantzler & Schmidt-Nielsen 1966). Much of the water, sodium and chloride are reabsorbed from the bladder (Stetson 1989), therefore urine

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Table 7.22 Endoparasites of chelonians, their diagnosis and significance.

Organism	Identification	Significance
Coccidia terrestrial chelonians—*Eimeria* and, less commonly, *Isospora* sea turtles—*Caryospora* and *Eimeria*	• *Eimeria* is characterised by ovoid, sporulated oocysts (10–15 µm × 25–37 µm) (Barnard 1986a) in faeces, each containing four spherical sporocysts. • *Isospora* is characterised by oocysts containing two sporocysts. • *Caryospora* oocysts contain a single large sporocyst and are sometimes elongate.	• Terrestrial chelonians are usually asymptomatic. • May contribute to debility in sick animals (Barnard 1986a). • Fatal ulcerative enteritis has been associated with *Caryospora cheloniae* in both wild and captive green turtles (*Chelonia mydas*) (Leibowitz *et al.* 1978; George 1997). • Sulpha drugs are effective against *Eimeria* but not *Caryospora*. Hygiene is important in control.
Intranuclear coccidiosis	• Intranuclear protozoa visible under light and electron microscopy in multiple tissues (Jacobson *et al.* 1994; Garner *et al.* 1998). Diagnosis in all but one case has been post mortem. • One affected animal was shown to have *Eimeria* oocysts in faeces (Jacobson *et al.* 1999b).	• Jacobson *et al.* (1994) described two cases in *Geochelone radiata*. Garner *et al.* (1998) reported on seven cases in various species. • At least in the cases described, this is a disease with high mortality and multi-organ involvement. Enteritis, otitis interna, nephritis, pancreatitis and pneumonitis all have been demonstrated.
Amoebae *Hartmanella* *Acanthamoeba* *Entamoeba* *Endolimax*	• An excellent summary of amoeba identification can be found in Telford (2000). Trophozoites (amoeboid form) have a single nucleus and average diameter of 16 µm when fixed. Cysts (Fig. 7.38) average 11–20 µm. Both are visible in wet faecal smears. With experience they can be detected in unstained preparations. Staining facilitates identification to genus. Lugol's iodine (ideally double strength), haematoxylin or merthiolate are suitable for this purpose. • *Hartmanella* spp. have large cysts in which the single nucleus contains an endosome occupying over one half of its diameter. • *Acanthamoeba* spp. have similar cysts but with an irregular outline. • *Entamoeba* spp. have multinucleate cysts in which the nuclear endosomes measure up to $^{1}/_{3}$ the diameter of the nucleus. • *Endolimax* spp. have multinucleate cysts in which the nuclear endosomes measure $^{1}/_{3}$ the diameter of the nuclei, each of which has a distinct, dark-staining rim. • In the amoebiasis cases described by Jacobson *et al.* (1983) no amoebae were detected in faeces. Histopathology of duodenum and liver were diagnostic. • Carrier chelonians may shed only small numbers of trophozoites. Such animals can be difficult to pick up on routine faecal or cloacal-flush samples but still represent a threat to other species in a collection. Cranfield & Graczyk (1999) suggest that multiple cloacal flushes be taken from each animal, and found that *in vitro* culture of samples prior to microscopy led to increased sensitivity.	• Although amoebae are often present in the faeces of chelonians they are usually considered to be non-pathogenic. • Chelonian amoebae may, however, act as a source of infection for snakes and lizards. • Pathogenic *Entamoeba* infestation has been reported in red-footed and leopard tortoises (*Geochelone carbonaria* and *G. pardalis*) (Jacobson *et al.* 1983; Jacobson 1994). Characterised by anorexia, malaise, diarrhoea and high mortality. • Sporadic single cases in other spp. (Keymer 1981) including captive sea turtles (George 1998). • It has been anecdotally reported that giant species are especially susceptible (Klingenberg 1993). • Dimetridazole or metronidazole in food appears to be an effective treatment for extra-intestinal amoebiasis.
Cryptosporidium	• Oocysts (~5 µm) are most easily detected using phase-contrast microscopy after faecal flotation. They must be differentiated from yeasts (Current 1999). Alternatively, wet faecal smears may be acid-fast stained (Cranfield *et al.* 1996) or acid-fast stained with brilliant green as counterstain (*Cryptosporidia* appear dark red on green background). Shedding may be intermittent. • A *Cryptosporidium*-specific ELISA test is available. • Immunofluorescence microscopy kits also exist.	• Reported in two testudinids, both suffering from gastritis and regurgitation (O'Donoghue 1995). Graczyk *et al.* (1998) reported the histopathological diagnosis of fatal cryptosporidiosis in one *Testudo kleinmanni*.

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Table 7.22 (*cont'd*)

Organism	Identification	Significance
Flagellates *Hexamita* *Trichomonas* *Monocercomonoides* *Tritrichomonas, etc.*	• Variable numbers of motile flagellated protozoa (approximately 8 × 5 µm) may be found in wet mounts of faeces. • Sometimes very numerous. Possibly commoner in sick animals. • In hexamitiasis, a six-flagellated (two caudal, four cranial) protozoa may be seen in urine or coelomic effusion (Zwart & Truyens 1975). Definitive ante-mortem diagnosis requires renal biopsy. • Flagellates are best identified in phase-contrast microscopy as the number of flagella or undulating membranes are then better discernible.	• Zwart & Truyens (1975) described fatal renal tubular hexamitiasis in eight chelonian species. Clinical signs were non-specific. • We have not yet diagnosed this disease in our patients at Holly House Surgery. It appears to be uncommon in the United Kingdom. • Significance of intestinal flagellates is controversial. Barnard (1986a) suggests that sick animals 'may suffer deleterious effects'. Bone (1992) attributes anorexia, diarrhoea and polydipsia to flagellate infestation. Telford (1971) stated that trichomonads should not be considered pathogenic. Subjectively it does seem that the faeces of sick animals often carry more flagellates. • Klingenberg (1993) notes that prophylactic treatment of box turtles with metronidazole at transportation 'dramatically improves survival rates'. This effect might equally be explained by amoebicidal activity. • Animals with clinically-apparent hexamitiasis also showed histopathological signs of enteritis (Zwart & Truyens 1975).
Ciliates *Balantidium* *Nyctotherus* (Figs 7.31, 7.34, 7.35 & 7.39)	• Cyst and ciliated (trophozoite) forms in faeces. • *Nyctotherus* is large (up to 250 µm) whilst *Balantidium* does not exceed 150 µm.	• Current consensus suggests that these organisms are probably commensal although a wide variety of contradictory opinions have been published (Bone 1992; Mader 1996). Some animals with gastrointestinal disturbances do have large numbers of ciliates in their faeces. • Cyst forms may be misidentified as parasites.
Ascarids turtles–*Sulcascaris* terrestrial chelonians–*Angusticaecum* (Figs 7.31–7.32)	• Thick-walled, round eggs in faeces often with scalloped surface usually contain embryo. 80–100 × 60–80 µm. • Endoparasiticide treatment revealed an incidence of ascarid infestation three times that revealed by multiple faecal flotations (Satorhelyi & Sreter 1993). A negative faecal exam should not be taken as a conclusive all-clear. • Adults are large roundworms–up to 10 cm long (Frank 1981).	• Direct and indirect life cycles (Barnard & Upton 1993). • Quite common in captive chelonians (21/70 animals in Holt *et al.* 1979 but only 8/71 in Satorhelyi & Sreter 1993). • Affected animals are usually asymptomatic. • May exacerbate concurrent disease. • Gastrointestinal obstruction (especially after anthelminthic treatment) is particularly likely in small individuals (Satorhelyi & Sreter 1993) and may lead to vomiting, anorexia or death. • Intussusception, gastrointestinal ulceration, coelomitis, thromboembolism and avascular necrosis have been associated with ascaridiasis (Frye 1991a).
Proatractis	• Viviparous—larvae in direct smears or Baermann apparatus preparation of faeces. Characterised by a rosette around the buccal opening (Caballero 1971). • At post mortem large numbers of 5–10 mm worms in caecum and colon.	• Specific to chelonians (Rideout *et al.* 1987). • Direct life cycle. • Non-specific clinical signs—anorexia and lethargy preceded the death of eight *Geochelone carbonaria* and three *G. pardalis* (Rideout *et al.* 1987). • Optimal treatment unknown. Most anthelminthics known to be ineffective. • Hygiene important.
Oxyurids (pinworms) *Ortleppnema* *Alaeuris Mehdiella* *Tachygonetria* *Thaparia* spp.	• Eggs (Fig. 7.33) are variable but lack scalloped surface and very thick walls typical of ascarids. • Often asymmetrical (D-shaped). • Some species oval or elongate. • May be operculate at one or both ends. • Larvae often also present. • Adults are small and may be present in vast numbers in the large intestine.	• Direct life cycle, host-specific (Telford 1971). • Common: 75% of our (RW & SM) patients have eggs in faeces. 50/71 captive tortoises in Hungary infested (Satorhelyi & Sreter 1993). 4/70 infested (Holt *et al.* 1979) in the United Kingdom. • Affected animals are usually asymptomatic. • Infestation may exacerbate concurrent problems. Frank (1981) observed that heavily-parasitised animals were prone to fatal post-hibernation anorexia.

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Table 7.22 (*cont'd*)

Organism	Identification	Significance
		• Klingenberg (2000) considered that infection may predispose to rectal prolapse. • Severe infestations may cause anorexia or obstruction.
Spirurids	• Dracunculid larvae may be present in skin samples (Frank 1981).	• Adults are present in the body cavity. Gravid females lie in subcutaneous tissue and shed larvae through the skin (Frank 1981). • Apart from skin lesions these infestations appear to be non-pathogenic.
Acanthocephalans (spiny-headed worms)	• Eggs are present in faeces and are said to be 'easily identified' (Frank 1981), however no description was offered. • Larvae may be found encapsulated in the intestinal wall or other viscera. • Adults occupy the intestine.	• Indirect life cycle. Mollusc intermediate hosts. Aquatic or semi-aquatic chelonians may be heavily infested. Martin (1972) found 123/125 *Trachemys scripta* to be affected. • No clinical signs reported (Frank 1981). Although Telford (1971) considered them to be potentially pathogenic.
Cestodes (tapeworms)	• Thick-walled, spherical eggs of *Ophiotaenia* have visible hooklets.	• Probably indirect life cycles. • Cestodes appear to be uncommon in chelonians (there appear to be no reports for sea turtles). *Ophiotaenia* occurs rarely in freshwater chelonians (Frank 1981). *Glossocercus* and *Bancroftiella* are cestode parasites of freshwater turtles (Pichelin *et al.* 1998). No pathogenic effects reported.
Trematodes (flukes) (Figs 7.40–7.42)	• Distinctive orange or deep-yellow thin-shelled, often operculated eggs in faeces. *Nyctotherus* (ciliate) cysts are also yellowish and operculate. Frank & Reichel (1977) suggest that cestode eggs may be similar in some cases. May contain discernible miracidia later in course of development. • Spirorchid eggs from sea turtles are elongate and have hooked terminal processes (George 1997). • Adult spirorchid trematodes are 1–2 mm long and inhabit the heart and blood vessels. They may be difficult to locate despite the presence of eggs within granulomatous lesions throughout the body (Johnson *et al.* 1998).	• Manfredi *et al.* (1998) found trematodes in 11/14 loggerhead turtles in the Adriatic sea. • Monogenetic (direct life cycle) trematodes inhabit the nasopharynx or urinary bladder of aquatic chelonians (e.g. Platt 2000; Du Preez & Lim 2000) and are probably non-pathogenic. • Digenetic trematodes (intermediate host necessary) may inhabit a variety of internal organs. In terrestrial and freshwater chelonians they are generally non-pathogenic. However, Johnson *et al.* (1998) described fatal trematodiasis in a captive population of the freshwater turtles *Trachemys scripta* and *Chrysemys picta*. Lethargy, constant swimming, sideways swimming, hemiplegia and ulcerative shell lesions were noted. Trematode eggs consistent with *Spirorchis* were found throughout the body and in faeces. • Marine turtles are commonly affected with spirorchids and may suffer serious vascular disease or pneumonia (Wolke *et al.* 1982).
Leeches	• May be present on freshwater or marine chelonians. Martin reported 15/287 animals of various freshwater species to be infested in Louisiana. *Ozobranchus branchiatus* is found on green turtles in the tropics while *O. margoi* is widespread, occurring on most species of sea turtle (George 1998).	• Restricted to aquatic chelonians. • George (1997) reported that severely affected marine turtles suffer extensive skin damage and anaemia. They can be removed by immersing the animal in fresh water for one hour.
Sarcophaga cistudinis	• The larvae of these flies are true parasites on various species of North American chelonians (Knipling 1937). Females are ovoviviparous. Maggots can be found on the exposed skin of the neck and limbs. In some cases the larvae may penetrate intact skin although wounds or tick bites are more readily infested.	• A dipteran parasite reported in the box turtle, gopher tortoise and Aldabran tortoise (Knipling 1937). The average number of larvae in each infestation was 16. Severely infested animals with over 100 larvae may die—presumably from overwhelming secondary bacterial infection.
Cloacal mites (Cloacaridae)	• Nymphs present in tiny pustules on the cloacal mucosa (Camin 1967).	• Reported in *Chelydra serpentina* and *Chrysemys picta* by Camin (1967) and subsequently in *Chelonia mydas* by Pence & Wright (1998). Probably pruritic!

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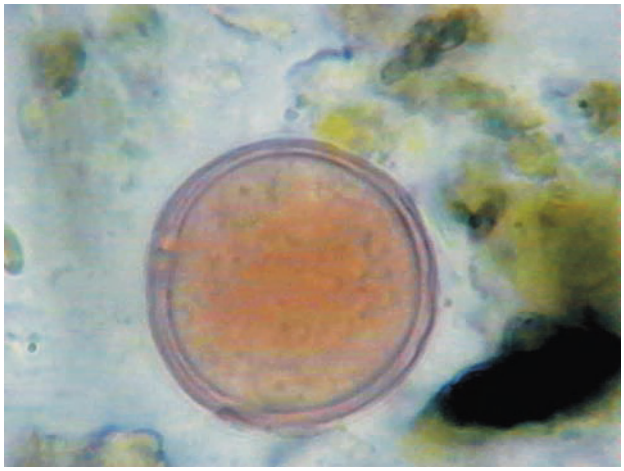

Fig. 7.38 An **amoebic cyst** in a faecal wet smear from a healthy *Geochelone sulcata*. Stained with Lugol's iodine; approximately × 1000 magnification.

Multiple nuclei, each with a single dark endosome, can be seen at 6 o'clock and 11 o'clock. Multinucleate cysts in which the nuclear endosomes are less than $^1/_3$ the diameter of the nucleus are characteristic of *Entamoeba*.

Fig. 7.39 An egg in a faecal wet smear from a healthy *Geochelone sulcata*. Stained with Lugol's iodine; approximately × 100 magnification.

This is probably a cyst of the ciliate *Nyctotherus*. Care is needed to distinguish from operculated, yellow-orange eggs produced by flukes—particularly in aquatic species.

Table 7.23 Electrolyte content (mEq/kg dry weight) of urinary solids in the desert tortoise (*Gopherus agassizii*) (Minnich 1972).

Na^+	K^+	NH_4^+	Cl^-
0.34	3.08	0.20	0.001

composition reveals little about renal function. Prange & Greenwald (1980) also showed that dehydration had little effect on urine electrolyte concentrations in marine turtles, since the salt gland appears to play the dominant role in homeostasis.

Innis (1997b) found that the specific gravity of urine from a variety of terrestrial herbivorous species maintained in captivity and apparently healthy varied from 1.003–1.012. In two animals emerging from hibernation (enforced dehydration) urine specific gravity was 1.008 and 1.012. Christopher *et al.* (1994) found

Fig. 7.40 Pharynx with **polystoma infestation.** *Emys orbicularis* with a massive infestation of monogenic trematodes of the genus *Polystoma* in the pharynx. Monogenic trematodes have a direct life cycle and are parasites of fish, amphibians and reptiles. Removal is done mechanically with a forceps.
(Courtesy of J. Meyer)

Fig. 7.41 ***Polystoma***: a monogenic trematode of the genus *Polystoma*. Monogenic trematodes are normally 2–3 mm in length. They are inhabitants of the upper respiratory and digestive tracts as well as the urinary bladder. 40 × magnification
(Courtesy of J. Meyer)

that, at peak rainfall in July, desert tortoises (*Gopherus agassizii*) produced urine with a specific gravity of 1.007 ± 0.002. At other times of the year urine was significantly more concentrated, rising to a maximum of 1.017 ± 0.012 at emergence from hibernation. It would seem that urine SG is a potentially useful indicator of hydration status in terrestrial chelonians.

pH

The pH range reported by Innis (1997b) for healthy, herbivorous, terrestrial chelonians was 8.0–8.5 and this appears to be true for our *Testudo* patients (Wilkinson & McArthur: unpublished data). In a limited number of animals we have also found this to be the case for healthy, omnivorous *Terrapene* spp. In immediate

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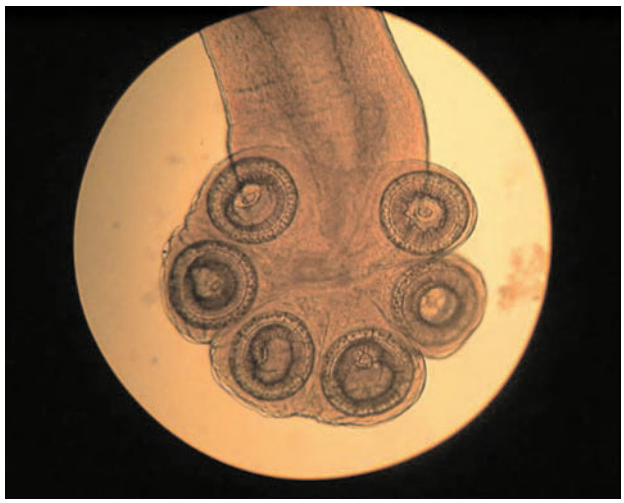

Fig. 7.42 ***Polystoma*** (Caudal end): Caudal area of a monogenic trematode of the genus *Polystoma*. Six suction cups at the rear end are typical for this genus. 100 × magnification
(Courtesy of J Meyer)

post-hibernation animals, Innis found that pH was 5.0 and 6.0 but this rose to 8.0 and 8.5 after one month of normal feeding. Acidic urine (< pH 7) is a consistent feature of herbivorous chelonians suffering from prolonged anorexia (Innis 1997: personal observation). The reason for this remains unknown.

As initially suggested by Innis (1997b), we have found that measurement of urine pH is a useful indicator of health status when dealing with an animal for which limited history is available. For example, post-purchase or post-importation, or when the individual is one of a larger group.

Ketones

Christopher *et al.* (1994) found that, in desert tortoises (*Gopherus agassizii*), urine beta-hydroxybutyrate levels were negligible in times of significant rainfall (and food availability) but increased to a maximum of 0.24 mmol/l in some animals after two months of drought. This phenomenon may in part account for the pH changes discussed above.

Protein

Protein levels of up to 30 mg/dl were recorded by Innis (1997b) in apparently healthy *Testudo graeca*.

Possible indicators of renal disease

Kölle & Hoffman (2002) found that the urine of tortoises suffering from renal disease (as indicated by blood samples and ultrasonography) exhibited significantly increased concentrations of AST, urea, calcium, CK, creatinine, glucose, LDH, ammonia and phosphorus than apparently healthy animals. The precise criteria used to define the renal disease group were not stated. However, any test which has the potential to improve our ability to diagnose renal insufficiency ante-mortem merits serious consideration.

Bacteruria is normal in chelonians. Because the urodeum is continuous with the coprodeum the bladder contents are potentially non-sterile and might contain elements of faeces even when sampled by cystocentesis. Innis (1997b) detected moderate numbers of bacteria in the voided urine of healthy tortoises.

The possibility exists that the techniques described by Dantzler & Schmidt-Nielsen (1966) for collection of ureteral urine might be applied for diagnostic as well as research purposes. These authors calculated clearance ratios after infusion of creatinine via a femoral vein catheter. It seems that, in tortoises from arid environments, glomerular filtration rate (as measured by creatinine clearance ratio) does not vary much in the face of dehydration (otherwise urates would precipitate in the tubules) and might provide an indication of renal function in such animals.

Biliverdinuria (green urine and urates) has been recorded in animals with erythrolysis or hepatic dysfunction (Figs 7.43–7.44). Uroliths of varying chemical composition have been recorded. On microscopic examination, the eggs of trematodes (rarely nematodes) which inhabit the urinary tract are very occasionally found in the urates. The presence of flagellates would be expected in renal hexamitiasis but such organisms may be present in the urine of normal animals due to faecal contamination.

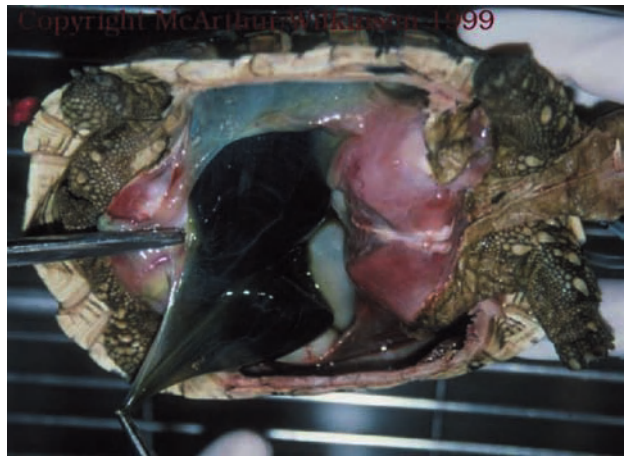

Fig. 7.43 Apparent biliverdinuria in a leopard tortoise (*Geochelone pardalis*) at post mortem. The bladder occupies the centre of the coelomic cavity and is filled with dark green urine.
This animal is one of a group with intra-erythrocytic inclusions which may have precipitated a haemolytic crisis. Biliverdin may be the principal haemoglobin breakdown product in reptiles.

Fig. 7.44 Green-stained urine from a Testudo tortoise with a severe hepatopathy. The green pigment is believed to be biliverdin.

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The bladder is lined with columnar epithelial cells, some of which may be ciliated. These cells secrete mucopolysaccharide into the urine, which protects the bladder wall from the abrasive urate crystals. The action of this mucociliary 'escalator' may help in the propulsion of crystals towards the cloaca.

HISTOPATHOLOGY

Tissue samples taken for histopathology should be preserved in a volume of buffered 10% formalin solution that is ten-fold greater in volume than the sample that is being preserved. Thin samples (ideally less than 1 cm) are less likely to undergo autolysis before formalin penetration. Freezing results in artefactual change and must be avoided.

TOXICOLOGY

Lead

A snapping turtle (*Chelydra serpentina*) that had ingested a lead fishing weight and exhibited clinical signs consistent with lead toxicosis was shown to have blood lead levels of 3.6 ppm (Borkowski 1997). After surgical removal of the sinker and treatment with calcium EDTA over a 40-day period, the lead level declined to below the limit of detection (0.05 ppm). In other species, blood lead concentrations > 0.6 ppm are considered strong evidence of lead poisoning. Levels below this do not preclude a diagnosis of lead toxicosis.

Heavy metals

Heavy metal and pesticide levels have been investigated primarily in marine turtles (Saeki *et al.* 2000). For heavy metal estimations, Jacobson recommended that at least 200 g of liver, kidney and skeletal muscle samples should be collected and stored in separate plastic bags before freezing.

Pesticides

For pesticide analysis, fat, liver, kidney and skeletal muscle are selected and wrapped in aluminium foil before storing in plastic bags and freezing. Sea turtles have been shown to have detectable levels of the organochlorines PCB and DDE in their eggs (Hillestad *et al.* 1974). Loggerheads (*Caretta caretta*) have consistently higher levels than green turtles (*Chelonia mydas*).

Oil and tar

Oil and tar may cause skin changes but exposure to crude oil also leads to changes in haematological parameters, glucose metabolism and salt-gland function (George 1997).

MICROORGANISMS

VIROLOGY

Clinical cases and pathological specimens are illustrated in Figs 7.46–7.72.

Strong evidence has been published for the existence of herpesvirus, toga virus, pox virus and iridovirus-related disease in chelonians (Jacobson *et al.* 1985; Sudia *et al.* 1975; Orós *et al.* 1998; Westhouse *et al.* 1996). To date, herpes-virus-related disease has apparently been seen in *Chelonia mydas, Graptemys pseudogeographica, G. barbouri, Clemmys marmorata, Chrysemys picta, Chersina angulata, Gopherus polyphemus, G. agassizii, Geochelone chilensis, G. pardalis, Homopus areolatus, Malacochersus tornieri, Testudo graeca, T. hermanni* and *T.* (*Agrionemys*) *horsfieldi*. All species should be regarded as potentially susceptible (Origgi *et al.* 1999). The existence of retroviruses in marine turtles has been suggested.

Suspicion of viral infection may be aroused by histopathological or cytological findings. The next step is generally to seek supportive evidence in the form of viral particles on electron microscopy. Samples for transmission electron microscopy (TEM) should generally be preserved in glutaraldehyde, but the institution that will be doing the microscopy should be contacted to confirm this. In some circumstances, formalin-fixed tissue can be used.

Fig. 7.45 Conjunctivitis in a juvenile *Testudo horsfieldi* from which *Mycoplasma agassizii* was isolated (personal data) Herpesvirus serology showed relatively high antibody titre against herpesvirus serotype II (Marschang 2000: personal communication). These eye lesions are typical of self-trauma from the scales on the inside of the forelegs.

This animal was one of a group of 24. Other animals in the group demonstrated profound stomatitis or rhinitis in addition to the conjunctivitis illustrated.

(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

Fig. 7.46 Rhinitis and conjunctivitis in *Testudo hermanni*. Herpesvirus infection, lytic agent × infection and iridovirus infection are associated with conjunctivitis, stomatitis and rhinitis in Mediterranean species.

(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

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Fig. 7.47 The same animal as in Fig. 7.26. Severe glossitis/stomatitis is present.
(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

Fig. 7.48 *Testudo horsfieldi* with conjunctivitis and rhinitis. *Mycoplasma agassizii* and herpesvirus were successfully isolated from this animal. It is possible that these agents act together to produce pathology and that clinical signs are most likely in the post-hibernation period when animals are immunocompromised and leucopaenic.
(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

Fig. 7.49 Diphtheritic membrane formation in a *Testudo graeca* with severe post-hibernation stomatitis. The external nares show depigmentation and inflammation associated with rhinitis. This is a chronic presentation.
(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

Fig. 7.50 Stomatitis, rhinitis and conjunctivitis (*Testudo graeca*). Here there is also a severe bacterial infection. This animal made an excellent recovery following placement of an oesophagostomy tube, analgesia, antibiosis, fluid, nutritional and environment support and Acyclovir therapy (200 mg/kg/day in divided doses through the oesophagostomy tube).
(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

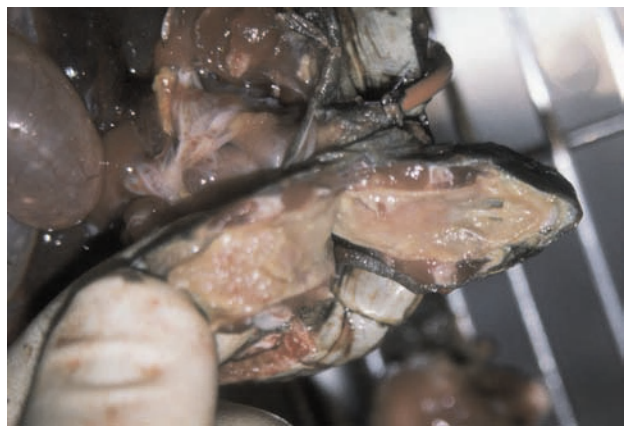

Fig. 7.51 Post-mortem examination of a *Testudo graeca* suffering from rhinitis, glossitis and pharyngitis. The choana is easily seen.
Extensive diptheritic membrane formation can be associated with herpesvirus infection. Intranuclear inclusions were seen on histopathological examination. This type of disease is commonly associated with herpesvirus, iridovirus or lytic agent × infection.
(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

Fresh or rapidly-frozen tissue samples are suitable for virus isolation. Alternatively, material from live patients may be inoculated into viral transport medium, after consultation with the laboratory. Herpesvirus may be easier to isolate from oropharyngeal swabs than conjunctival or cloacal (i.e. faeces/urine) samples (Marschang *et al.* 1997a). Viraemic animals may also have demonstrable virus in blood samples (Marschang *et al.* 1997). Unfortunately, isolation of reptilian viruses is limited by the availability of suitable cell lines, although some research institutes have projects underway in the United Kingdom and Europe (herpesvirus was first successfully isolated in a turtle embryo cell line by Kabisch & Frost 1994). Commercial lines are available in the United States (Jacobson 1992).

Molecular diagnostic tests are potentially sensitive and specific tools in our diagnostic armoury. Although such tests are in their infancy, the likelihood that their availability will greatly increase

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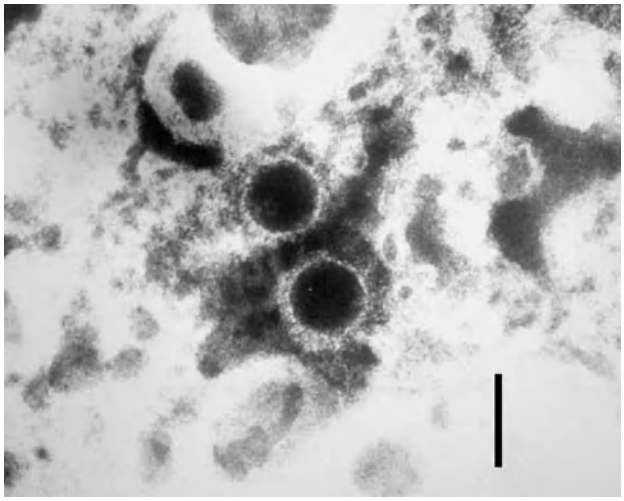

Fig. 7.52 Negative-contrast electron micrograph of herpesvirus particles from homogenate of the spleen of a *Testudo hermanni* with lymphoproliferative disease. Capsomeres can be seen at the particle perimeter.
Magnification × 38 000
Bar = 100 nm
(Courtesy of Sally Drury, Avian Virology, Veterinary Laboratories Agency)

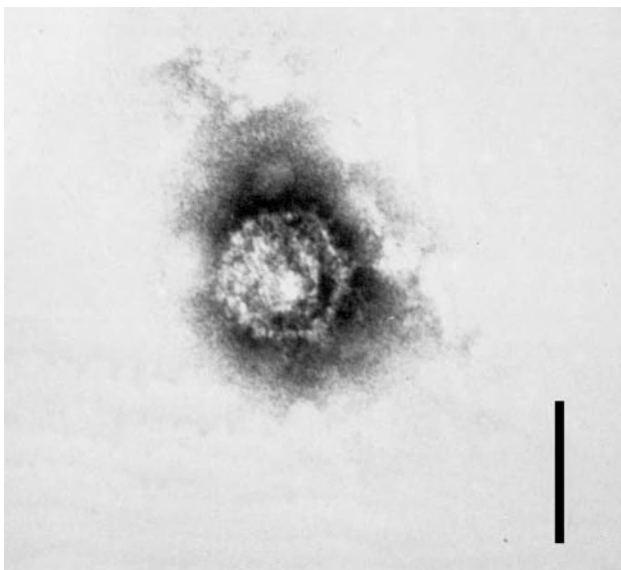

Fig. 7.53 Electron micrograph of a herpesvirus particle from the liver of *Testudo hermanni*. Icosahedral symmetry and some of the visible surface structure are typical of herpesvirus morphology.

To achieve a diagnosis of virus-associated disease in reptiles, viral agents may be isolated in cell lines, histopathology may demonstrate inclusion bodies, serology (VN or ELISA) may demonstrate viral neutralising antibodies. Immunohistochemistry of cytology or histopathology samples, or further molecular tests such as PCR may demonstrate the presence of virus.
Magnification × 38 000
Bar = 100 nm
(Courtesy of Sally Drury, Avian Virology, Veterinary Laboratories Agency)

in the future should encourage workers to preserve specimens in anticipation of using these powerful tools retrospectively. Serodiagnostic tests for specific antibody in blood samples require (ideally monoclonal) anti-chelonian immunoglobulin antibodies and a source of appropriate antigen.

Herbst *et al.* (1995) reported the production of monoclonal antibodies against *Chelonia mydas* immunoglobulin classes.

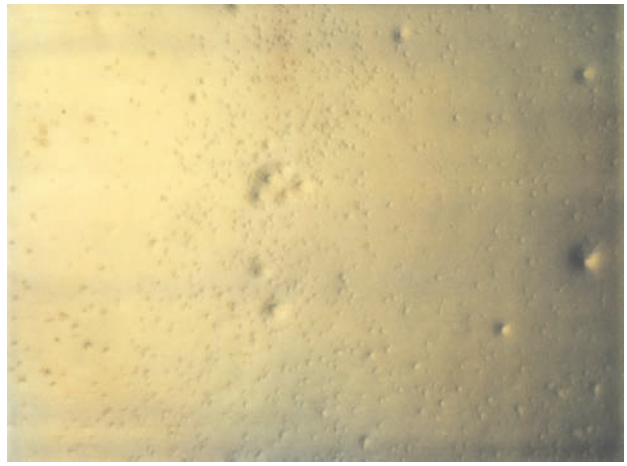

Fig. 7.54 *Mycoplasma agassizii* colonies isolated from United Kingdom captive *Testudo horsfieldi* with upper respiratory tract disease and conjunctivitis (like Figs 7.45–7.46). The agent was isolated from both eyes and choana of affected animals, which also showed relatively high antibody titre against herpesvirus serotype II (Marschang 2002: personal communication).
(Courtesy of Helena Windsor, Mycoplasma Experience, United Kingdom)

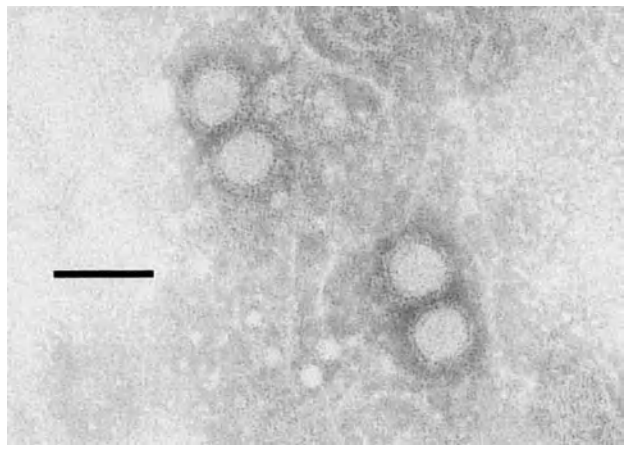

Fig. 7.55 Flavivirus isolated from *Geochelone pardalis* with fatal disease associated with haemolysis and wasting.
Magnification × 38 000
Bar = 100 nm
(Courtesy of Sally Drury, Avian Virology, Veterinary Laboratories Agency)

Kabisch & Frost (1994) developed a neutralisation test for herpesvirus and demonstrated rising titres in affected *Testudo hermanni* and *T. horsfieldi*. Marschang *et al.* (1997a) also demonstrated anti-herpes-virus antibody in healthy *T. graeca* in contact with affected *T. hermanni*. Work is under way in the development of both ELISA and immunoperoxidase-based serological tests and an immunohistochemical technique for the detection of herpesvirus antigen in tissue samples (Origgi 1999; Origgi & Jacobson 1999). A polymerase chain reaction (PCR) assay has been developed for the detection of fibropapilloma-associated herpesvirus in marine turtles (Jacobson 2000) and herpesvirus stomatitis of tortoises (Une *et al.* 1999). A PCR assay is now available in Europe and North America for the detection of chelonian herpesvirus infection (Origgi 2002: personal communication; Marschang 2002: personal communication). Table 7.23 summarises the diagnostic tests and their appropriate sample collection protocol.

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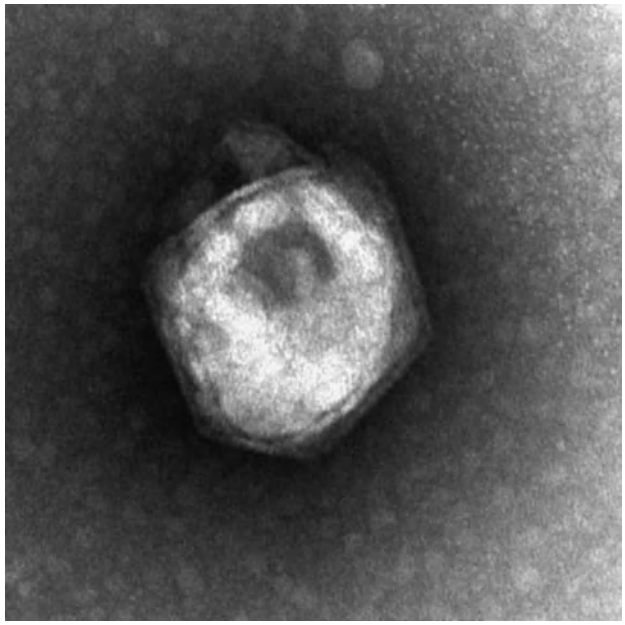

Fig. 7.56 Negative-contrast electron micrograph of iridovirus particle isolated from a *Testudo hermanni* with systemic disease associated with stomatitis, enteritis and hepatitis.
Magnification × 50 000
(Courtesy of Dr. Rachel E. Marschang, Institute for Environmental and Animal Hygiene, and Dr. Michael Ibsch, Institute for Zoology, Hohenheim University, Germany)

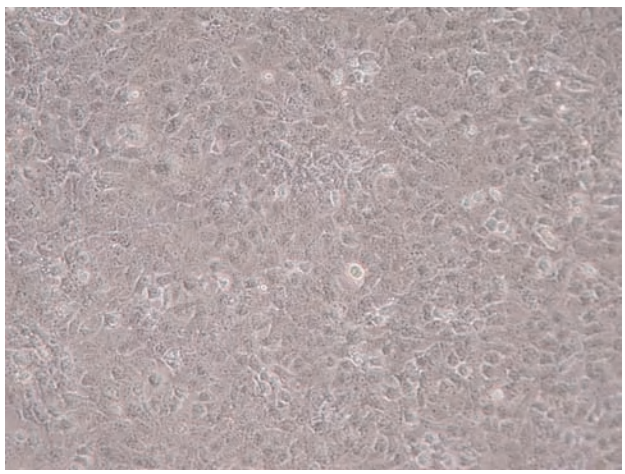

Fig. 7.57 *Terrapene* heart cell monolayer (TH-1, ATCC, Manassas, VA, USA). This cell line is commonly used to isolate chelonian viral agents. Shown is a normal monolayer with no cytopathic effect.
(Courtesy of Dr. Rachel E. Marschang, Institute for Environmental and Animal Hygiene, Hohenheim University, Germany)

According to Jacobson (1997), more cases of herpesvirus infections are documented in chelonians than in all other types of reptiles. Viral infections of chelonians by non-herpes viral agents are also well described in the literature. Non-herpesviruses intimately associated with disease include iridovirus (Heldstab & Bestetti 1982; Müller *et al.* 1988; Westhouse *et al.* 1996; Marschang *et al.* 1998a; Marschang *et al.* 1998b; Marschang *et al.* 1999; Cunningham 2000), papilloma-like virus (Jacobson *et al.* 1982b), and pox virus (Orós *et al.* 1998). Marschang *et al.* (1999) and Cunningham (2000) report the isolation of an antigenically

Table 7.23 Investigation of suspected viral disease.

Test	Sample collection
Histopathology	10% buffered formalin
Electron microscopy	Ideally glutaraldehyde or Trump's solution. Formalinised tissue may be suitable.
Virus isolation	Frozen or fresh (ideally in virus transport medium) tissue
Molecular diagnostic tests (e.g. southern blotting or PCR)	Deep frozen (–70°C for those requiring non-degraded DNA/RNA) or formalinised

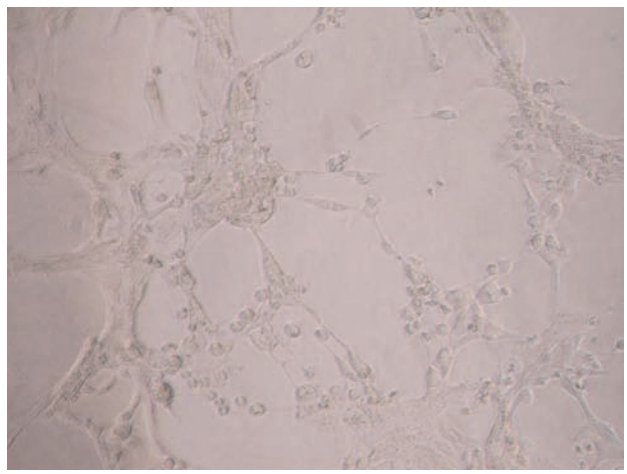

Fig. 7.58 Cytopathic effect of a chelonian herpesvirus, isolated from an oral swab from a *Testudo horsfieldi* with stomatitis, on TH-1cells at seven days post inoculation.
Magnification × 100
(Courtesy of Dr. Rachel E. Marschang, Institute for Environmental and Animal Hygiene, Hohenheim University, Germany)

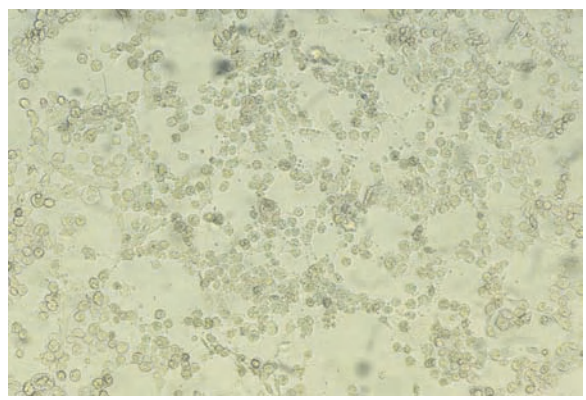

Fig. 7.59 Cytopathic effect of a chelonian iridovirus, isolated from the tongue of a *Testudo hermanni* with systemic disease associated with stomatitis, enteritis and hepatitis, on TH-1 cells at five days post inoculation.
Magnification × 100
(Courtesy of Dr. Rachel E. Marschang, Institute for Environmental and Animal Hygiene, Hohenheim University, Germany)

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similar iridovirus (ranavirus) from both diseased frogs and tortoises in Europe; Cunningham (2000) suggests that this virus might also affect fish in garden ponds in the United Kingdom. Table 7.24 below summarises probable pathogenic viruses.

Some viral agents have been observed in chelonians, or isolated from chelonians, but their association with disease in the field is unclear. These include a reovirus (Marschang *et al.* 1998a), an unidentified agent described as *lytic agent* (Marschang *et al.*

Table 7.24 Some viral agents implicated as probable pathogens of chelonians.

Virus family	Species affected	Disease	References
Herpesvirus	*Chelonia mydas*	Grey-patch Disease (GPD)	Rebell *et al.* (1975) Haines & Kleese (1977)
	Chersina angulata *Gopherus agassizii* *Geochelone chilensis* *Geochelone pardalis* *Homopus areolatus* *Malacochersus tornieri* *Testudo graeca* *Testudo hermanni* *Testudo horsfieldi*	Necrotic Stomatitis *Maulseuche* (German) (upper digestive tract disease) Occasionally, further signs such as rhinitis and systemic disease are also described.	Biermann and Blahak (1994) Harper *et al.* (1982) Jacobson *et al.* (1985) Cooper *et al.* (1988) Braune *et al.* (1989) Lange *et al.* (1989) Müller *et al.* (1990) Oettle *et al.* (1990) Kabisch & Frost (1994) Pettan-Brewer *et al.* (1996) Marschang *et al.* (1997a) Marschang *et al.* (1997b) Muro *et al.* (1998a) Drury *et al.* (1999 a & b) Marschang *et al.* (1999) Origgi (1999) Une *et al.* (1999) Teifke *et al.* (2000)
	Chelonia mydas	Lung, Eye, Trachea Disease (LETD)	Jacobson *et al.* (1986) Curry *et al.* (2000)
	Graptemys pseudogeographica *Graptemys barbouri*	Hepatitis, systemic disease, malaise and death	Jacobson *et al.* (1982b)
	Chelonia mydas	Fibropapillomatosis	Jacobson *et al.* (1991a) Quackenbush *et al.* (1998) Lackovich *et al.* (1999) Lu *et al.* (2000) Yu *et al.* (2000 & 2001)
	Lepidochelys olivacea	Fibropapillomatosis	Brown *et al.* (1999) Lackovich *et al.* (1999)
	Caretta caretta	Fibropapillomatosis	Lackovich *et al.* (1999)
	Clemmys marmorata *Chrysemys picta*	Systemic disease, malaise and death	Frye *et al.* (1977) Cox *et al.* (1980)
	Testudo hermanni *Geochelone pardalis*	Lymphoproliferative disease	McArthur (1998) Drury *et al.* (1998)
Papilloma virus	*Platemys platycephala*	Cutaneous lesions	Jacobson *et al.* (1982a)
Iridovirus	*Testudo hermanni*	Necrotic Stomatitis Rhinitis, pneumonia Systemic disease	Müller *et al.* (1988) Marschang *et al.* (1998a) Marschang *et al.* (1998b) Cunningham (2000)
	Gopherus polyphemus	Pharyngeal abscess	Westhouse *et al.* (1996)
	Testudo hermanni	Systemic disease	Heldstab & Bestetti (1982)
	Trionyx sinensis	Red-neck disease	Chen *et al.* (1999)
Pox virus	*Testudo hermanni*	Cutaneous lesions	Orós *et al.* (1998)
Sinensis virus	*Trionyx* spp.	Lethal disease	Zhang-QiYa *et al.* (2000)

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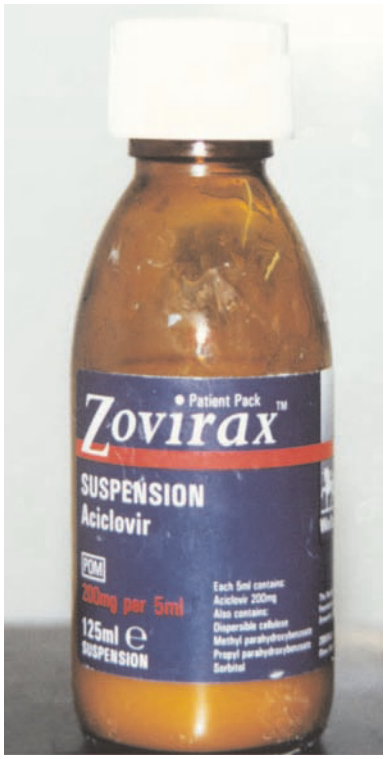

Fig. 7.60 This author (SM) has observed subjectively positive responses in chelonian patients suffering from stomatitis, when treated with acyclovir (200 mg/ml/day) (Zovirax ®, Wellcome).

A dose rate of 80 mg/kg has been advised in earlier texts, but this may be sub-therapeutic (McArthur 2000b). The author now employs a dose of 30 – 80 mg/kg three times daily by stomach or oesophagostomy tube.

Monitoring blood levels would be a method of ensuring therapeutic levels (contact local hospitals). No adverse reactions have been noted in approximately 100 animals treated.
(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

Fig. 7.61 Nasal flush.

Upper respiratory tract disease can be managed in some cases by treatment directly into the nasal chambers. The needle is removed from the hypodermic leaving the short, blunt hub that fits easily into the nares and allows flushing with fluids, dilute antibiotics or other medications. Flushing should be performed with the head tilted downwards to avoid aspiration of infectious material.
(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

Fig. 7.62 Dry-eye associated with herpesvirus during an episode of lymphoproliferative disease in a colony of *Testudo hermanni.* A transient whitening of the cornea occurred in several animals early in the course of the disease.
(Courtesy of the *Journal of Herpetological Medicine and Surgery*)

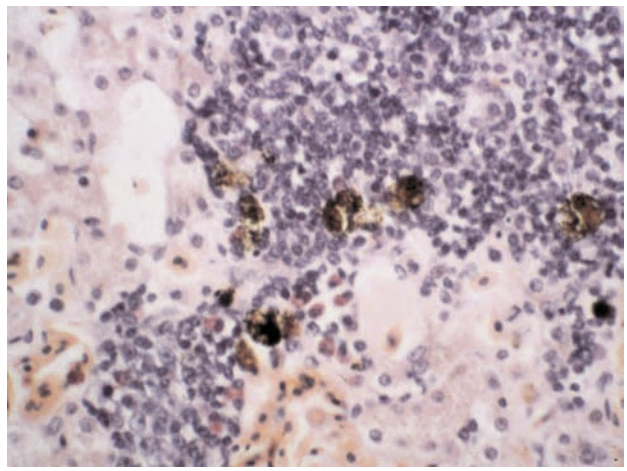

Fig. 7.63 Hepatic histopathology associated with lymphoproliferative disease (magnification × 400).

Predominantly lymphocytic infiltrates were present in large groups in portal tracts and around dilated hepatic veins with smaller groups in sinusoids. The lymphocytes were mixed large and small in type but small cells predominated and mitotic figures were rare. Occasional heterophils were also present within infiltrates.
(Courtesy of Dr. Joan Rest, DipECVPath MRCVS)

1998a) and a papilloma-like virus (Drury *et al.* 1998). Casey *et al.* (1997) present evidence of retroviral infection in normal *Chelonia mydas* turtles and those with fibropapillomatosis. Reports describe: the experimental infection of *Testudo (Agrionemis) horsfieldi* with an avian retroviral agent (Svet-Moldavsky *et al.* 1967); the experimental infection of *Mauremys mutica* and *Chinemys reevesii* with human hepatitis B virus (HBV) (Lee & Yoo 1989); the multiplication of sheep abortion virus in lung tissue culture of *Testudo graeca* (Shindarov & Vassileva 1966); the multiplication of variolo vaccine virus in *Testudo graeca* pancreas tissue culture (Shindarov & Vassileva 1969); the multiplication of Myxovirus parainfluenzae 1 (Sendai) in *Testudo graeca* lung tissue culture (Shindarov *et al.* 1969). Biermann (1995) describes the isolation of a paramyxovirus from a snapping turtle but gives no evidence of pathogenicity. Table 7.25 summarises these viruses.

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Table 7.25 Further possible pathogenic viruses.

Virus family	Species affected	Disease	References
Retrovirus	*Testudo horsfieldi*	Experimental retroviral infection resulting in tumour formation.	Svet-Moldavsky *et al.* (1967)
	Chelonia mydas	Natural retroviral infection of turtles with and without fibropapillomatosis.	Casey *et al.* (1997)
Reovirus	*Testudo graeca*	Virus was isolated from the tongue, oesophagus, lung and kidney of a diseased *Testudo graeca*.	Marschang *et al.* (1998a)
Papilloma virus	*Testudo horsfieldi*	This tortoise had stomatitis. Viral findings may have been incidental.	Drury *et al.* (1998)
Lytic agent	*Testudo graeca* *Geochelone pardalis* *Testudo hermanni*	Not specified. This agent was isolated when diseased individuals were undergoing investigation for the presence of virus. Clinical signs were stomatitis and upper respiratory tract disease (Marschang 1999: personal communication).	Marschang *et al.* (1998a)

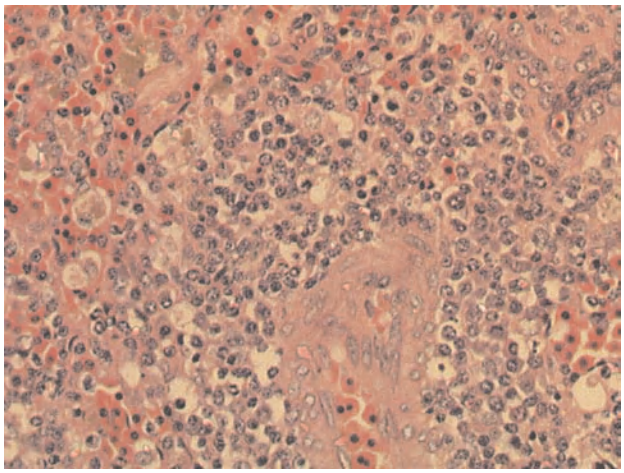

Fig. 7.64 Splenic histopathology associated with lymphoproliferative disease (magnification × 400).

Mixed populations of mononuclear cells obliterated the distinction between red and white pulp. These cells included both mature and immature lymphocytes and tangible macrophages. The mitotic rate was moderate. Heterophils were rare in infiltrates.
(Courtesy of Dr. Joan Rest, DipECVPath MRCVS)

Fig. 7.65 *Geochelone pardalis* near death. Herpesvirus and adenovirus were isolated from this tortoise. Alpha-flavivirus and herpesvirus were isolated from other members of the group. The disease presented with biliverdinuria, wasting and episodes of haemorrhage, prior to death.

Fig. 7.66 Neurological signs in juvenile *Geochelone pardalis*. This animal repeatedly smashed its head against the table top in frenzied feeding behaviour whilst retaining its forelimbs in flexion. Its pupils were wildly dilated and it appeared to be unaware of its surroundings. Intraerythrocytic inclusions were plentiful and flavivirus and other cytopathic agents were isolated from this and other affected animals, following euthanasia and post mortem examination.

Fig. 7.67 A second individual from the same group as the tortoise in Fig. 7.66, showing identical neurological signs associated with the presence of similar intraerythrocytic inclusions.

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Fig. 7.68 Ticks are associated with transmission of viral and other infectious agents, such as flavivirus and *Plasmodium* sp. Ticks were present in several of the animals in a group of *Geochelone pardalis* affected by severe haemolytic disease, where flavivirus was isolated. It is not clear however that this was a causative agent in the disease observed.

Fig. 7.69 Ocular and dermal lesions associated with fibropapillomatosis in a juvenile green turtle (*Chelonia mydas*). Turtles presenting with dermal lesions may also suffer visceral lesions. Extensive assessment should be considered prior to embarking upon surgical therapy and rehabilitation.

There are a number of reports of viral disease where chelonians are regarded as an intermediate or reservoir host of a disease pathogenic to another species but not the chelonian host. These include eastern equine encephalitis virus (EEEV) and western equine encephalitis virus (WEEV). Some of the papers tabulated below (Table 7.26) suggest that such viral infections may overwinter within reptilian species.

BACTERIOLOGY

Samples for bacteriology should be inoculated into transport medium for both aerobic and anaerobic culture. Tissue samples and biopsies from which bacterial isolation is to be attempted should also be embedded in medium. Bacteria, particularly anaerobes, are most easily isolated from effusions and exudates when a swab is immersed in the sample and then transferred to transport medium.

Some knowledge of the normal bacterial flora of chelonians is vital to the interpretation of bacteriology results. Some data on the normal flora of the respiratory tract and oral cavity are available (Snipes *et al.* 1980; Lawrence & Needham 1985; MacDonald 1998). Without this data, the causative role of any bacterial isolates from non-sterile sites (respiratory tract, lower urinary tract, skin and gut) remains largely speculative.

Fig. 7.70 Close up of the lesions of the same animal as in Fig. 7.71.

Fig. 7.71 Lesions of fibropapillomatosis in marine turtles develop rapidly and may affect sight and limb use. This can prevent normal feeding and render the animal vulnerable to predation.

Histopathological or cytological evidence of pathogenicity is important. Control samples from healthy animals are very useful. Samples should be tested for aerobes *and* anaerobes. Stewart (1990) found that 54% of laboratory submissions for bacteriology from sample collection sites such as abscess/fibriscesses, lungs and coelomic cavity yielded anaerobes. This finding is important since aminoglycosides are ineffective against such pathogens.

Mixed bacterial infections are common. Figure 7.27 illustrates a cytological preparation from a lung abscess from which *Paecilomyces, Citrobacter* and *Bacteroides* were cultured. Blood cultures may be useful in animals with suspected septicaemia although it may not be easy to distinguish contaminants from genuine pathogens.

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Table 7.26 Viral infections where chelonians appear to act as an intermediate or reservoir host, but no chelonian pathogenicity was observed (Ahne 1993; Shortridge 1989).

Virus family	Virus	Species	Method	Author
Toga virus	EEEV	*Chrysemys picta* *Clemmys guttata* *Terrapene carolina* *Kinosternon subrubrum*	INF, AB	Dalrymple *et al.* (1972)
	EEEV	*Chelydra serpentina* *Terrapene carolina*	INF, AB, IS	Dalrymple *et al.* (1972)
	EEEV	*Kinosternon subrubrum*	INF, AB	Karstad (1961)
	EEEV	*Gopherus polyphemus* *Trionyx ferox*	INF	Karstad (1961)
	EEEV	*Clemmys insculpta*	INF	Hayes *et al.* (1964)
	WEEV	*Chrysemys picta* *Clemmys guttata* *Kinosternon subrubrum*	INF, AB INF, AB	Dalrymple *et al.* (1972) Smith & Anderson (1980)
	WEEV	*Chelydra serpentina* *Terrapene carolina*	INF, AB, IS	Dalrymple *et al.* (1972)
	WEEV	*Gopherus berlandieri*	INF, IS	Bowen (1977); Sudia *et al.* (1975)
	WEEV	*Chrysemys scripta*	AB	Hoff & Trainer (1973)
	WEEV	*Malaclemys terrapin*	IS	Goldfield & Sussmann (1964)
	WEEV	*Trionyx spiniferus*	IS, AB	Hoff & Trainer (1973)
Flavivirus	SLEV POWV	*Emydidae* *Chrysemys picta*	AB	Whitney *et al.* (1968)
	POWV	*Chelydridae* *Chelydra serpentina*	AB	Whitney *et al.* (1968)
	JEV	*Trionychidae* *Trionyx sinensis*	AB	Shortridge *et al.* (1975)
Rhabdovirus	VSVG	*Trionyx spiniferus*	AB	Cook *et al.* (1965)
Bunyavirus	BUNV	*Emydidae* *Chrysemys picta*	AB	Whitney *et al.* (1968)
	BUNV	*Chelydra serpentina*	INF, IS, AB	Dalrymple *et al.* (1972)
	BUNV	*Trionyx spiniferus*	IS, AB	Hoff & Trainer (1973)
	CCHFV	*Testudo horsfieldi*	AB	Pak *et al.* (1975)

[Key]

EEEV	Eastern Equine Encephalitis Virus	INF	Experimental infection
WEEV	Western Equine Encephalitis Virus	IS	Isolation of virus
SLEV	St. Louis Encephalitis Virus	AB	The presence of antibodies to the virus
POWV	Powassan Virus	JEV	Japanese Encephalitis Virus
CCHFV	Crimean Congo Haemorrhagic Fever Virus		
VSVG	Vesicular Stomatitis Virus Group		
BUNV	Bunyamwera Virus		

MYCOPLASMATA

Mycoplasmata have been isolated from the airways of desert tortoises (*Gopherus agassizii*), Horsfield's tortoises (*Testudo horsfieldi*) and leopard tortoises (*Geochelone pardalis*) with respiratory disease (Brown *et al.* 1994; Lederle *et al.* 1997; McArthur *et al.* 2002a). Mycoplasmosis is also reported as having been 'identified' in gopher tortoises (*Gopherus polyphemus*), Indian star tortoises (*Geochelone radiata*), Asian tortoises (*Indotestudo* spp.), spur-thighed tortoises (*Testudo* spp.) and box turtles

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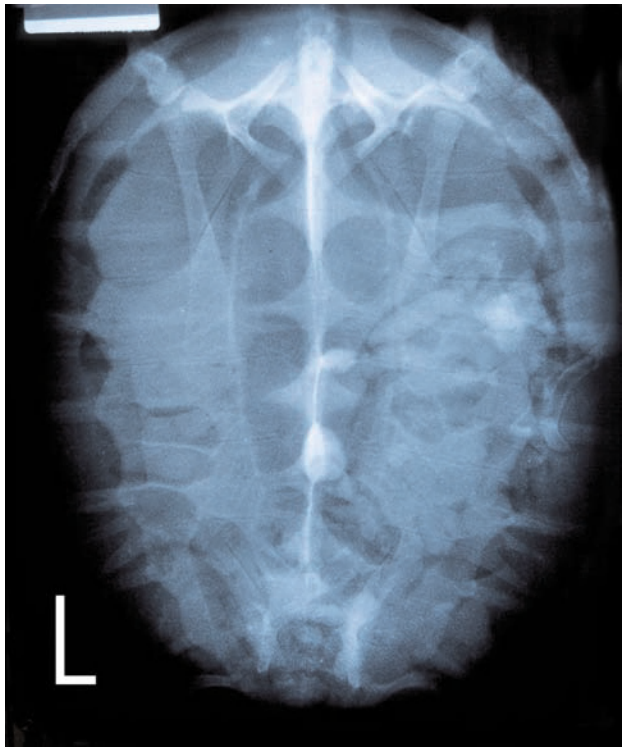

Fig. 7.72 Turtles presented with dermal lesions of fibropapillomatosis at Marathon Turtle Hospital, Florida, are examined internally using radiography and endoscopy for evidence of visceral lesions. Two radio-opaque masses consistent with visceral fibropapillomas are apparent in this turtle radiograph.
(Courtesy of Marathon Turtle Hospital)

(*Terrapene carolina bauri*) (Origgi 1999). All species should be regarded as potentially susceptible. McArthur *et al.* (2002b) also demonstrated that mycoplasmata could be isolated from *Geochelone pardalis* two months after the resolution of signs of respiratory disease. It is likely from such preliminary findings, that an asymptomatic carrier state is common. A polymerase chain reaction (PCR) survey of both healthy and sick captive *Testudo* spp. in the United Kingdom revealed that *Mycoplasma agassizii* was common in the study population, regardless of health status (SM: unpublished data).

Mycoplasma culture, detection of anti-mycoplasma antibodies (by ELISA) and PCR detection of mycoplasma gene sequences have been used in the diagnosis of mycoplasma infection (McLaughlin *et al.* 2000). ELISA testing is sensitive but does not distinguish past exposure from current infection and there is the potential for false-positive results though cross reaction with antibody to similar bacterial antigens.

Samples for culture, taken using viscose-tipped swabs, can be inoculated into mycoplasma experience (ME) transport medium (McArthur *et al.* 2002a). Bacterial overgrowth from oral samples was less of a problem when patients had not eaten on the day of sampling. The specialist laboratory used by McArthur *et al.* found that growth on ME solid medium was typically found in 14–21 days. Liquid media proved advantageous only when dealing with small conjunctival samples. Identification (*M. agassizii* or *M. testudinis*) was confirmed by indirect immunofluorescence. MICs for doxycycline, enrofloxacin and tylosin should be sought.

MYCOLOGY

Various fungal commensals and potential pathogens have been isolated from reptiles (Austwick & Keymer 1981). Respiratory system, gut, skin and subcutaneous tissue are most often involved although mycosis of other viscera is sometimes seen. *Aspergillus, Fusarium, Geotrichum, Paecilomyces, Candida* and various Phycomycetes were the fungi most commonly identified as reptile pathogens by Migaki *et al.* (1984). Yeasts most commonly isolated by Kostka *et al.* (1997) included *Candida* (32/56 isolates), *Torulopsis* (9/56), *Rhodotorula* and *Trichosporon*. These were commoner in herbivorous chelonians.

Heard *et al.* (1986) describe a case of hyalohyphomycosis in an Aldabran tortoise (*Dipsochelys elephanina*). Hyalohyphomycosis encompasses opportunistic infections caused by non-pigmented fungi such as *Aspergillus*, which are characterised by septate, hyaline, hyphal elements. Fungi isolated from non-sterile sites should not necessarily be regarded as pathogens. Cultural isolation of these organisms is common, although cytological or histopathological evidence of mycosis is much less so. Fungal disease appears to be uncommon in wild animals and may be intimately related to the captive environment (Jacobson 1994).

MYCOBACTERIA

Mycobacterial infections are not uncommon in chelonians. These organisms require special attention because of their zoonotic potential and resistance to conventional therapy. Advance planning at the time of sampling is necessary where such infections are possible if the need for a second biopsy procedure is to be avoided.

Samples from a lesion should be divided into three parts: the first part should be used to prepare a slide for cytology and then preserved in formalin for histopathology; the second should be inoculated into transport medium for aerobic and anaerobic culture; the third should be stored in sterile saline for specific mycobacterial culture if histopathology or cytology suggest that this is a possible diagnosis. Any such samples should be handled with extreme respect for their zoonotic potential.

The correct protocol for Ziehl-Neelsen staining of acid-fast bacilli (as described by Needham 1981) is as follows:

(1) Flood smear with carbol fuchsin and warm until steam rises for ten minutes without allowing smear to dry out.
(2) Wash with distilled water.
(3) Decolourise with 20% sulphuric acid until the smear is a faint pink.
(4) Flood with Loeffler's methylene blue for 30 seconds, wash and dry. Acid-fast bacilli are bright red against a pale blue background.

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8 DIAGNOSTIC IMAGING TECHNIQUES

ROGER WILKINSON, STEPHEN HERNANDEZ-DIVERS, MAUD LAFORTUNE, IAN CALVERT, MICHAELA GUMPENBERGER AND STUART MCARTHUR

Diagnostic imaging techniques are of major importance in the assessment of chelonian health. Table 8.1 summarises these techniques.

ULTRASONOGRAPHY

(Roger Wilkinson)

Ultrasonography allows us to visualise chelonian organs, which are concealed beneath the shell and cannot be palpated or easily seen on radiography. It is an invaluable tool for the chelonian clinician.

APPARATUS

Whitaker & Krum (1999) recommend the use of a 3.5 or 5 MHz curvilinear transducer for examination of the coelomic cavity of adult marine turtles. Only sector scanners allow adequate visualisation of the internal organs through the restricted scanning windows of many smaller tortoises. A small transducer is highly desirable. With our 2.8 cm diameter probe we can scan Mediterranean *Testudo* tortoises down to 700 g via the cervical fossa and down to 1 kg via the more confined inguinal fossa. It should be noted that powerful animals could inflict significant damage on a transducer trapped between shell and hind limb. Frequencies of 5–10 MHz (usually best with 7.5 or 10) provide adequate depth of penetration and definition for chelonians weighing less than 4 kg.

A linear array was used in sea turtles by Rostal *et al.* (1990) and Swiss workers used a 5.0 MHz linear array for scanning the coelomic cavity of giant species of 77–114 kg (Casares *et al.* 1997). In giant tortoises, 5.0 MHz transducers give useful images of the reproductive tract but 3.0–3.5 MHz may be better for survey of other internal organs. 7.5 MHz transducers are not

Table 8.1 Summary of diagnostic imaging techniques.

Ultrasonography	Two acoustic windows allow inspection of medium-sized chelonians (>700 g) using a 3.5–10 MHz probe, depending on animal size. • The prefemoral acoustic window is of great use in the assessment of reproductive state in mature females, hepatic size and homogeneity, renal size and homogeneity. • The cervicobrachial acoustic window allows visualisation of the heart, liver, thyroid and great vessels.
Radiography	Three views enable survey assessment of the chelonian. • The dorsoventral (DV) view allows assessment of bone structure, the digestive tract, and the urogenital system–with limitations. • The craniocaudal view allows comparison of the lung fields and assessment of the volume of coelomic viscera. • The lateral view allows spatial determination of lesions identified in the other two views.
Endoscopy	Rigid and flexible endoscopes can be used to examine chelonians. • Coelomic endoscopy through the prefemoral fossa of an anaesthetised chelonian can allow inspection of the reproductive organs, liver and digestive tract. • The kidneys can be approached and biopsied through the prefemoral fossa (using a 30° oblique scope in most cases). • The lungs can be examined by a soft-tissue approach through a prefemoral coeliotomy at the level of the septum horizontale, or through a temporary transcarapacial osteotomy. • The upper respiratory tract can be examined via the choana through an oesophagostomy incision, or, in most adults, using a fine, 1 mm or 1.3 mm semi-rigid scope via the nares. • The upper digestive tract, oesophagus, stomach, pharynx and trachea can be reached by the oral route. Fine 1.3 mm scopes can also enter the bronchi. • The cloaca, lower digestive and urogenital tracts can be examined via the cloaca.
Magnetic resonance imaging (MRI)	This technique has already demonstrated excellent diagnostic potential in chelonians.
Computed tomography (CT)	Axial, sagittal or reconstructed horizontal scans allow differentiation of skeletal and soft tissues without superimposing structures. CT provides: • best visualisation of the typical reticular lung patterns and lung disorders; • sufficient information about liver, gall bladder, kidneys, follicles and eggs as well as urinary bladder and partly the heart; • three-dimensional reconstructions for surgical planning or teaching.

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suitable for coelomic ultrasonography in large species (Schildger *et al.* 1994).

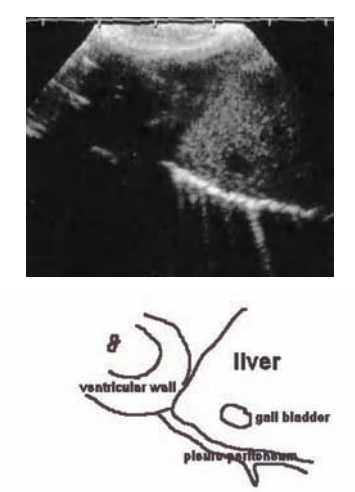
The use of Doppler ultrasound (to determine egg viability and to assess circulatory function in animals over 500 g body weight) is in its infancy (Highfield 1996; Redrobe 1997; Redrobe & Scudamore 2000).

EXAMINATION TECHNIQUE

Anatomical differences between species have a profound effect upon ease of scanning. For example Mediterranean tortoises (*Testudo* spp.) and soft-shelled turtles (*Trionyx* spp.) have relatively open cervical and inguinal fossae, while Horsfield's tortoises (*Testudo horsfieldi*) and red-footed tortoises (*T. carbonaria*) have narrow openings and are therefore more difficult to scan. Species with absent or incomplete ossification of the shell, such as soft-shelled turtles, can be examined ultrasonographically through the plastron (Figs 8.1–8.2a).

Fig. 8.1 Trans-plastral ultrasonography of a soft-shell turtle (Trionyx sp.).

Dorsoventral flattening of the body means that only a small section of the coelomic cavity can be visualised at any one time.



Figs 8.2 & 8.2a Ultrasonographic image of the liver of a soft-shell turtle (Trionyx sp.) as seen through the plastron.

The right of the picture is occupied by the homogeneous liver. The gall bladder is visible as a small, hypoechoic structure. To the left is the poorly-defined heart. The echogenic pleuroperitoneal membrane l ies dorsally.

Most chelonians can be examined with a little (sometimes more!) patience without sedation. The assistance of a second person to restrain the patient facilitates ultrasonography, although this is not essential. It seems likely that sedation or anaesthesia will influence cardiac measurements. It is easier to scan cool, soporific animals so the procedure should be scheduled for before rather than after periods of basking.

An animal balanced on top of a small prop, such as a tablet pot, so that its legs do not reach the ground, requires less restraint and leaves a hand free to press buttons. Fractious animals sometimes require head or forelimb restraint to get a particular view, but for most animals we have found that it generally involves less struggle in the long term to scan without head or leg restraint. Redrobe (1997) preferred an assistant to extend the head and legs.

This author (RJW) has found it easiest to apply the transducer directly to the skin (with a coupling gel) although it is also possible to immerse the animal and transducer in water. This is an alternative where the animal is too small to allow transducer access to the scanning windows, and has also been recommended for debilitated marine turtles (Whitaker & Krum 1999). With healthy sea turtles the latter authors still prefer, however, to scan out of water. Non-waterproof transducers can be wrapped in an examination sleeve. It is also possible to apply a water-filled glove to the requisite part of the animal and scan through this. Species with deep and narrow scanning windows (e.g. *Terrapene* spp.) can be held with the relevant acoustic window facing upwards. The hollow is then filled with coupling gel to which the probe is applied. Freshwater turtles, such as *Pseudemys* (*Trachemys*) and soft-shelled turtles (*Trionyx* spp.) (which are potentially dangerous to the operator) are best scanned partially submerged (Schildger *et al.* 1994).

Schildger *et al.* (1994) describe the approach to scanning giant terrestrial species. Both Aldabran and Galapagos tortoises will respond to gentle tactile stimulation of their soft parts by extending their neck and limbs, raising the body clear of the ground. In this position the mediastinal and inguinal shell openings are accessible for scanning.

Table 8.2 summarises the technique and uses of the main acoustic windows.

Cervicobrachial acoustic window

Similar information can be gained from left and right sides (Fig. 8.3a). The left atrium of Mediterranean tortoises is usually easier to visualise from the left, but when it can be seen from the right it is easier to obtain a reproducible M-mode picture through the maximum diameter. The same applies in reverse for the right atrium.

Thyroid

The cranial opening of the coelomic cavity is bounded on either side by the large, intensely echogenic scapulae and associated shoulder muscles, such as the large latissimus dorsi. Behind these, the thyroid gland is a good, relatively echogenic landmark in Mediterranean tortoises.

Visibility of the thyroid depends upon the degree of neck extension. It lies in the midline, ventral and cranial to the heart. Although Penninck *et al.* (1991) found that it could not be seen in desert tortoises (*Gopherus agassizii*). In most captive animals

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Table 8.2 The cervicobrachial and prefemoral acoustic windows.

Acoustic window	Technique	Advantages
Cervicobrachial (Figs 8.3–8.3a)	Pull forelimb laterally. Head is often best left unrestrained so long as patient will extend neck. Apply probe to junction of neck and forelimb. Angle beam parallel to plastron.	Allows visualisation of the thyroid, heart, liver, gall bladder and stomach.
Prefemoral (Figs 8.4–8.4a)	Pull hind limb caudally. Apply probe to fossa. If carapace restricts access, fill fossa with gel or immerse in water. Angle dorsally for kidneys.	Allows visualisation of the bladder, gonads, oviduct and kidneys.

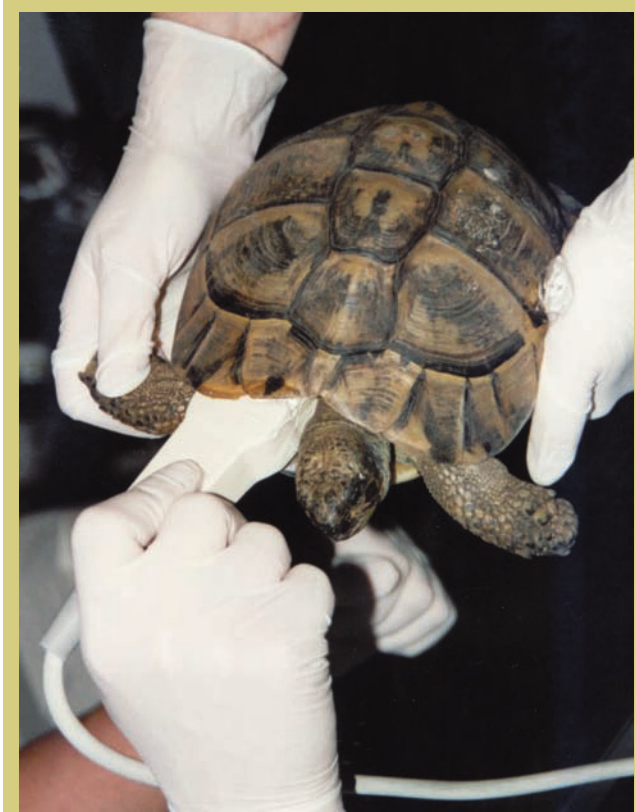

Fig. 8.3 Ultrasonographic examination of a Testudo sp. via the cervicobrachial acoustic window.

Most tortoises can be scanned without chemical restraint. It is useful to have an assistant to restrain one front leg. Holding the head usually leads to struggling.

Fig. 8.4 Ultrasonographic examination of a *Testudo* sp. via the prefemoral acoustic window.

An assistant restrains the hind limb.

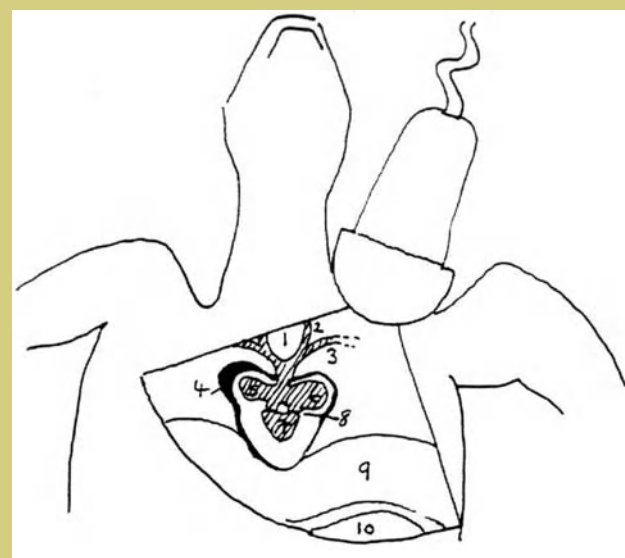

Fig. 8.3a Ultrasonographic view of the cranial coelomic cavity in a tortoise via the cervicobrachial acoustic window. The important viscera are seen in the frontal plane immediately above the plastron.

1) thyroid, 2) carotid artery, 3) subclavian artery, 4) pericardial space (may contain fluid), 5) right atrium, 6) left atrium, 7) ventricle, 8) intensely echogenic atrioventricular valves, 9) liver, 10) stomach.

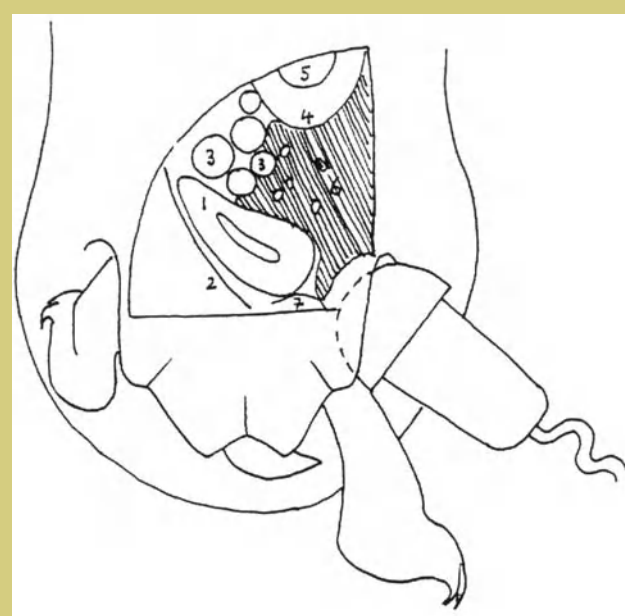

Fig. 8.4a Ultrasonographic view of the caudal coelomic cavity of a female turtle via the prefemoral acoustic window. Due to shell anatomy, the plane of scanning is normally oblique rather than in the frontal plane. This mealhat only one kidney is visualised at any one time.

1) kidney, 2) carapace, 3) ovarian follicles, 4) egg with calcified shell, 5) yolk, 6) urinary bladder containing hypoechoic urine and intensely echogenic urate crystals, 7) leg musculature.

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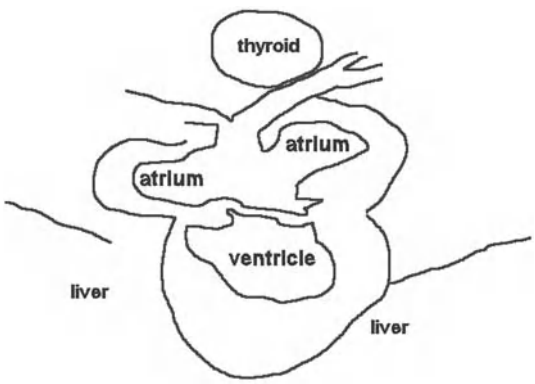
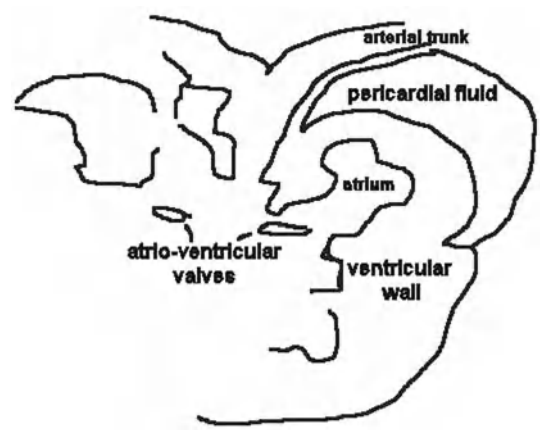


Figs 8.5 & 8.5a Ultrasonographic image of the cranial coelomic cavity in a healthy adult Testudo sp., seen from the cervicobrachial scanning window, showing the thyroid and heart.

The single ventricle is separated from the two atria by two echogenic valves. The major vessels cranially diverge at the caudal border of the thyroid. The thyroid is round to drop-shaped and homogeneous.



Figs 8.6 & 8.6a Ultrasonographic image of the heart in a healthy adult Testudo sp., seen from the cervicobrachial scanning window.

The left atrium and the left subclavian-carotid trunk are visible. Considerable pericardial fluid is visible. This is common in both sick and healthy animals. It should not be assumed to be pathological without cytological or histopathological evidence.

Blood flows slowly in chelonians and has slight echogenicity.

this organ is well defined, homogeneous and oval- to tear-drop-shaped. In adult Mediterranean tortoises with a variety of medical conditions (n = 12), thyroid varied in length from 8–14 mm and width from 6–13 mm. It is bounded caudally on both sides by the diverging, hypoechoic subclavian/carotid arterial trunks. Hypothyroidism/hypoiodinism caused by ingestion of goitrogens has been described in captive tortoises (Frye & Dutra 1974); these animals had enlarged thyroids. Thyroid adenoma and adenocarcinoma have been reported from chelonians (Machotka 1984; Cowan 1968).

Heart

The heart lies immediately above the plastron ventrally, and caudal to the thyroid. Its beating (10–20 beats per minute in adult *Testudo*) should be readily apparent. The left and right atria are of similar size with thin walls (1–3 mm in adult *Testudo*) and are separated from the single, thick-walled (5–7 mm in adult *Testudo*) ventricle by very echogenic atrioventricular valves, whose motion is often clearly discernible (Figs 8.5–8.5a).

Many tortoises appear on ultrasound to have a pericardial effusion (Figs 8.6–8.6a) (Wilkinson: unpublished observations). This may be a normal finding and should not be assumed to be pathological without supporting cytological or histopathological evidence. Jessop (1999: personal communication) reports the ultrasonographic detection of pericardial fluid in a healthy tortoise, which on analysis proved to have the characteristics of a modified transudate. In this animal, at coeliotomy, the left pericardial space was found to be continuous with the coelomic cavity. The same correspondent has also seen clear pericardial fluid as an incidental finding on coeliotomy in another patient. An earlier author (Darwin 1845) describes how in the Galapagos archipelago 'the inhabitants . . . always first drink the water in the pericardium, which is described as being best'.

In contrast however, Redrobe & Scudamore (2000) describe a case in which ultrasonographically visible pericardial fluid was accompanied by histopathologically-confirmed fibrinous pericarditis. These authors suggest that congestive heart failure may have resulted in pericardial effusion although this would not appear to offer a ready explanation for the inflammatory reaction. In summary, the significance of pericardial effusions in general remains open to debate. Where clinically appropriate, efforts should be made to acquire an aspirate of any visible fluid (trans-plastron or via the cervical fossa) for cytological examination.

Blood, which flows more slowly through the heart and large vessels than in mammals, is finely echogenic in most chelonians. The interior of the ventricle is contoured by the ridges, which partially separate the left and right cavities of the ventricle. In a single case, hyperechoic foci were visible in the wall of the left

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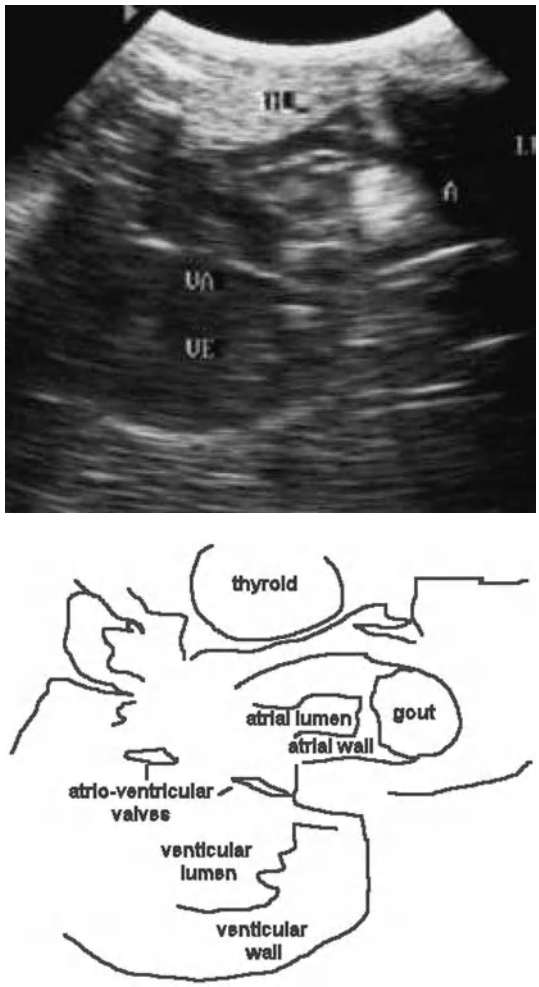


Figs 8.7 & 8.7a Ultrasonographic image of the heart in a sick adult Testudo sp., seen from the cervicobrachial scanning window.

The thyroid is visible. Below this is the contracted left atrium in the wall of which is a rounded echogenic lesion subsequently confirmed to be myocardial gout.

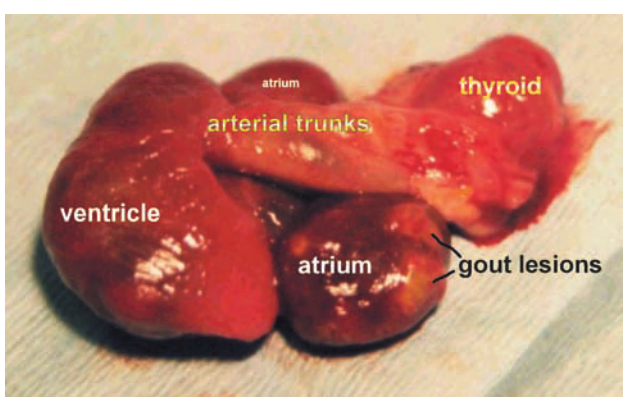
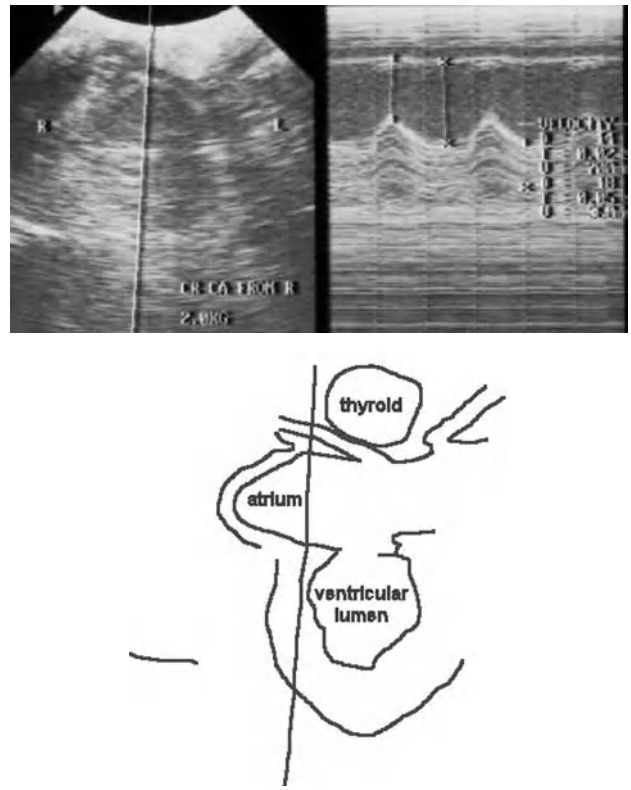


Fig. 8.8 Post-mortem appearance of myocardial gout, as demonstrated ultrasonographically in Fig. 8.7.

atrium (Figs 8.7–8.8). Histopathological findings were consistent with myocardial gout (urate deposits). Non-suppurative myocarditis, bacterial endocarditis and amoebic myocarditis have also been described in chelonians (Innis *et al.* 2002).

Because of restricted access points, it is difficult to obtain a meaningful M-mode image of ventricular activity that might



Figs 8.9 & 8.9a B-mode (right) and M-mode (left) images of the chelonian heart as seen via the left cervicobrachial acoustic window.

The M-mode image is taken from a line across the right atrium at its greatest craniocaudal diameter. In this way a consistent indication of cardiac function can be obtained.

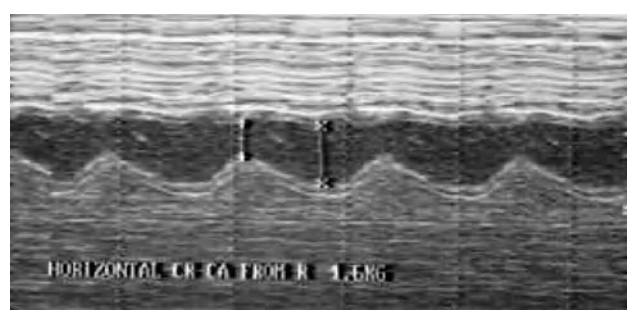


Fig. 8.10 M-mode image of the heart taken as described for Fig. 8.7 but across the left atrium as seen from the right cervicobrachial window.

Fractional shortening in healthy, unsedated Testudo spp. is typically 30%–50%.

provide a means to measure contractility and cyclic changes in wall thickness. The alignment of the atria, however, allows reproducible data to be collected from an M-mode image obtained from a frontal plane image with a line of scan across the greatest diameter of the left or right chamber, perpendicular to the linear division between atria and ventricle (Figs 8.9–8.10). Data from a number of animals has been collected according to this method, which appears to offer some promise for assessment of cardiac function.

Measurements were taken from the leading edges of the structures involved, as is conventional (i.e. measure from the outer margin of the atrial wall cranially to the inner margin of the atrial wall caudally). Left atrial craniocaudal diameter measured in this

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way in long-term captive Mediterranean (*Testudo*) tortoises with a variety of medical conditions ranged from 8–11 mm in systole and 12–18 mm in diastole. Right atrial craniocaudal diameter in the same hospital population was between 9–14 mm in systole and 13–19 mm in diastole (Wilkinson: unpublished data).

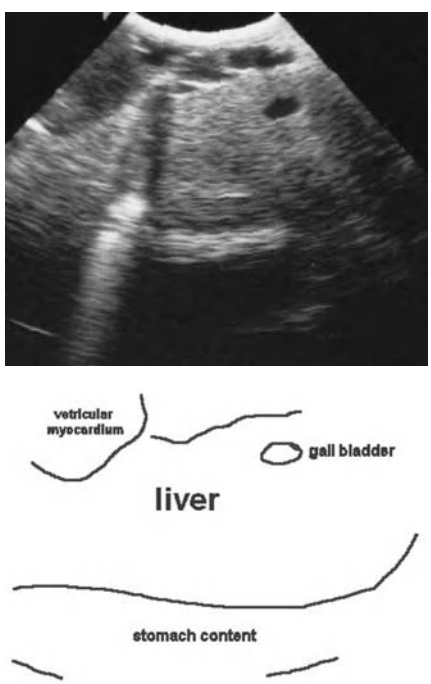
All but one of these animals was unsedated; the exception was recovering from propofol-induced anaesthesia. Figures for fractional shortening derived from this data ranged from 33%–47% using the left atrium and 31%–57% for the right atrium—broadly comparable to ventricular contractility in cats and dogs.

Vessels

The right and left aortae and the pulmonary trunk can be distinguished. The right aorta runs cranially a short distance before giving rise to a large trunk, which immediately bifurcates into the paired subclavian/carotid trunks at the caudal border of the thyroid. The left aorta curves around caudodorsally without branching and the pulmonary trunk curves from right to left before turning dorsal to the aortae and bifurcating. In marine turtles, ultrasonography can be a valuable aid in localising neck veins for venepuncture or catheterisation (Whitaker & Krum 1999).

Liver and gall bladder

The liver is located caudally to the heart and extends transversely like a curtain across the ventral half of the coelomic cavity (Figs 8.11–8.11a). The liver is recognised as a well-defined, homogeneous and moderately echodense tissue. Both left and right lobes are identifiable. They are wider (craniocaudally) laterally and narrow to the point of contact, just left of midline. The cranial and caudal borders of the liver are well defined near the midline but become difficult to visualise laterally. The thin-walled, round and hypoechoic gall bladder is consistently present in the right lobe near its caudal border but the exact position is variable.



Figs 8.11 & 8.11a Ultrasonographic image of the liver in a healthy adult Testudo sp., seen from the cervicobrachial scanning window.

At present, no objective criteria for the assessment of liver size and shape are available and such judgements remain subjective. Changes in appearance might be expected in hepatic lipidosis or viral hepatitis. Lipidosis in cats is associated with increased echogenicity (Center *et al.* 1993) and this also seems true of reptiles (Redrobe 1997; Divers & Cooper 2000). Irregularities due to abscessation, parasitism or neoplasia would be expected to be visible. A single case of hepatic neoplasia has been reported in a chelonian (Effron 1977), however various plates show hepatic neoplasia within this text (Figs 8.12–8.14).

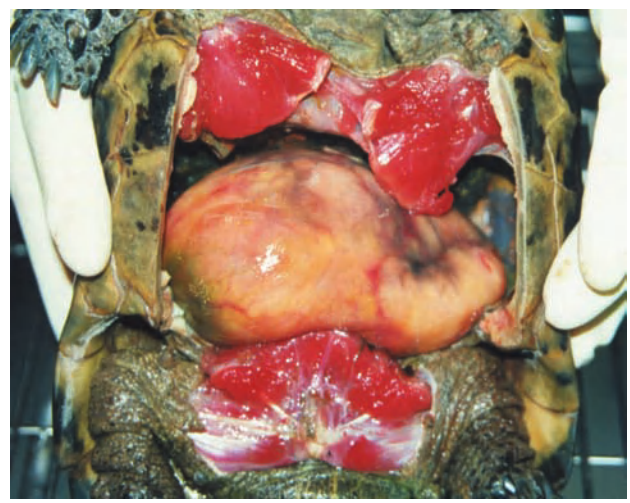


Fig. 8.12 Close up of the liver tumour in Fig. 6.34.

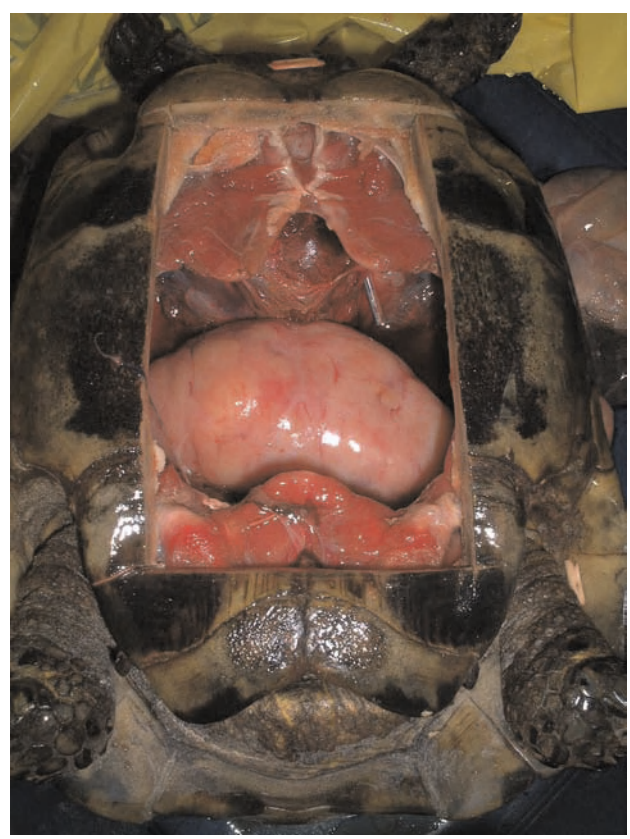


Fig. 8.13 Mature male Turkish spur-thighed tortoise (*Testudo graeca ibera*).
A large coelomic tumour was apparent upon ultrasonographic examination.

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Fig. 8.14 The same animal as Fig. 8.13.
The right testis was grossly abnormal and contained a carcinoma.

Gonads
Spherical, homogeneous, developing, pre-ovulatory follicles are often visible immediately caudal to the liver in adult females. Vast numbers may be present in ovarian stasis.

Gastrointestinal tract
The stomach lies left of midline immediately caudal and dorsal to the left lobe of the liver. The duodenum courses ventrally from left to right across the caudal border of the liver. These organs are discernible when fluid filled but prevent penetration of ultrasound waves when containing gas. In a situation where it became desirable to image the stomach, then gas might be withdrawn by stomach tube and water instilled. A gastric carcinoma has been reported in the black side-necked turtle (*Pelusios subniger*) (Cowan 1968).

Further structures might be visible under some circumstances although we have not yet confidently identified them. The spleen and pancreas lie right of midline, immediately caudal to the liver in the curve of the duodenum at the centre of the coelomic cavity. The spleen is ovoid and relatively small (1–2 cm long) in Mediterranean species. The normal pancreas of a Mediterranean tortoise is elongate, very narrow and a few centimetres long.

Thymus bodies are small (5 mm diameter), near-spherical bodies, and lie near the aortic arches and bronchi, dorsal to the heart. Parathyroid glands may also be present at various sites along the neck where it enters the shell. Spontaneous hyperparathyroidism has been described by Frye (1991a).

Axillary acoustic window

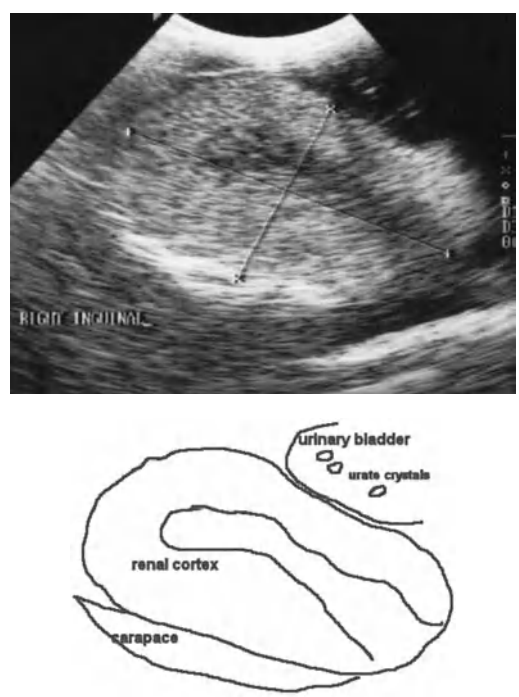
In marine turtles, Whitaker & Krum (1999) describe the use of the space between forelimb and plastron ventrally to scan the liver, heart and pectoral muscles. Ultrasound-guided liver biopsy can be achieved via the right axillary window. The axillary window is too confined in most terrestrial chelonians and freshwater turtles to be of use.

Prefemoral acoustic window

Access cranial to the hind limb is more restricted than in the cervical fossa and depends upon the shell architecture of the individual (Fig. 8.4a). In smaller or heavy-shelled animals (especially those with flared posterior marginal scutes) it may only be possible to get a single view at 45° to the horizontal, obliquely across the coelomic cavity.

Bladder
The size and shape of the bladder, and accessory bladders, is extremely variable. When full it may appear to fill much of the visible coelomic cavity and may be difficult to distinguish from coelomic effusion. The almost anechoic urine is normally patterned with intensely hyperechoic urate crystals. Calculi may be visible. The bladder wall is normally very thin.

Kidneys
The kidneys lie in a retrocoelomic position, close to the carapace and caudodorsal to the gonads and bladder (Figs 8.15–8.17a). Both kidneys are triangular-oval with a thicker 'head' and tapering 'tail'. In Mediterranean tortoises, kidney length varied between 24–37 mm and maximum width ranged from 13–18 mm. The centre of the kidney is often hypoechoic and the remainder coarsely patterned, variably but more or less homogeneously echogenic. Urate deposition or mineralisation of kidney tissue is not uncommon. A renal adenocarcinoma with liver metastasis was reported in the box turtle (*Terrapene carolina*) by Machotka (1984).



Figs 8.15 & 8.15a Ultrasonographic image of a normal kidney in a healthy Testudo as seen from the prefemoral acoustic window with the probe directed dorsally to the maximum extent.
The hypoechoic medulla is visible in this plane of scanning. The kidney lies adjacent to the intensely echogenic carapace and pleuroperitoneal membrane.

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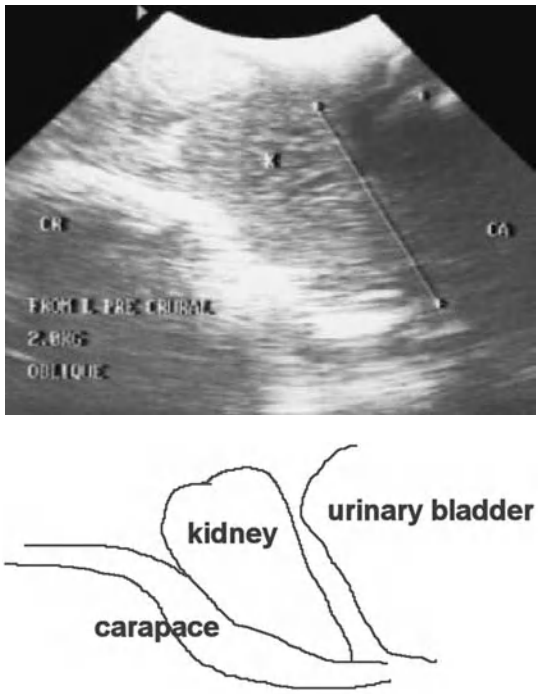
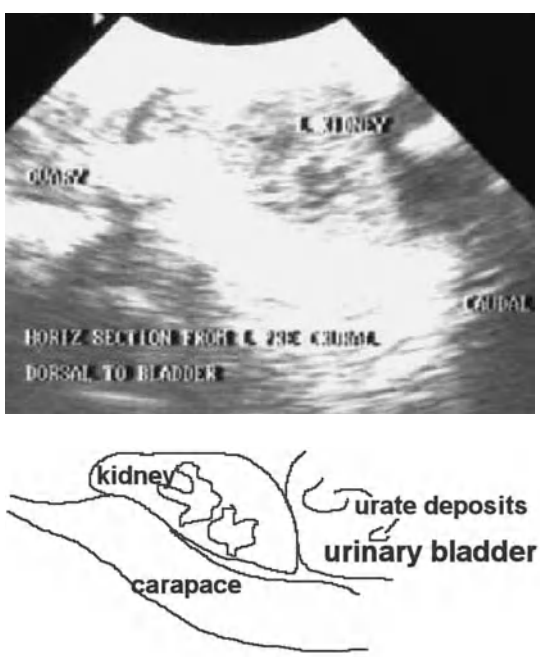


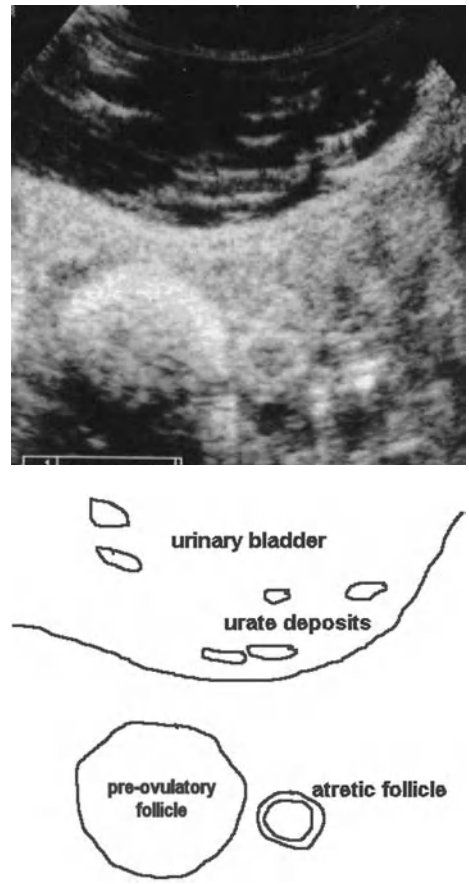
Figs 8.16 & 8.16a Ultrasonographic image of a normal kidney in a healthy Testudo as seen from the prefemoral acoustic window.
The rounded, elongate triangular profile is characteristic.



Figs 8.17 & 8.17a Ultrasonographic image of a kidney (upper right of image) in a Testudo with renal gout, as seen from the prefemoral acoustic window.
Urate deposits in both kidney and bladder are intensely echogenic.

Reproductive tract

Ovarian tissue is loosely arranged in the ventrolateral part of the coelomic cavity, extending from the liver to the bladder neck. Its visibility depends on the degree of follicle development. Follicular development, ovulation, albumen deposition and shell formation



Figs 8.18 & 8.18a Ultrasonographic image of the caudal coelomic cavity in a healthy adult female *Terrapene ornata* in summer.
The upper half of the image is occupied by the urine-filled bladder. Echogenic urate crystals are a normal finding. The lower left quadrant contains a large pre-ovulatory follicle (seen as a homogeneous sphere). Immediately to its right is a small atretic follicle (a 'hollow' ring).

may progress at remarkable speed—Casares *et al.* (1997) did not observe the albumen deposition phase at all in their study.

Vitellogenic follicles (Figs 8.18–8.18a) are spherical, homogeneous, echodense structures of up to 25 mm in diameter in Mediterranean tortoises and up to 42 mm in giant species (Casares *et al.* 1997). They may fill a significant part of the coelomic cavity and vast numbers may be present in ovarian stasis. Ultrasonography is probably the most important tool in the diagnosis of this common and life-threatening condition. Atretic follicles become patchily hypoechoic and sometimes anechoic with apparent sediment. Casares *et al.* (1997) reported that atresia may occur at any stage of follicular development and reported sizes up to 67 mm in *Chelonoids nigra*.

Corpora lutea have been described as spherical fluid-filled structures of up to 13 mm in diameter (Swingland & Coe 1978). It is not clear whether these might be ultrasonographically distinguishable from atretic follicles. Non-mineralised ova within the oviduct are of similar echodensity to vitellogenic follicles but are enclosed within a pocket of fluid. They become surrounded by hypoechoic albumen and subsequently by the eggshell.

Mineralised eggs are readily visualised (Figs 8.19–8.19a) and their contents, echodense yolk and hypoechoic albumen, are also discernible in Mediterranean tortoises. It might be possible to determine egg viability *in utero*. The oviduct is sometimes visible

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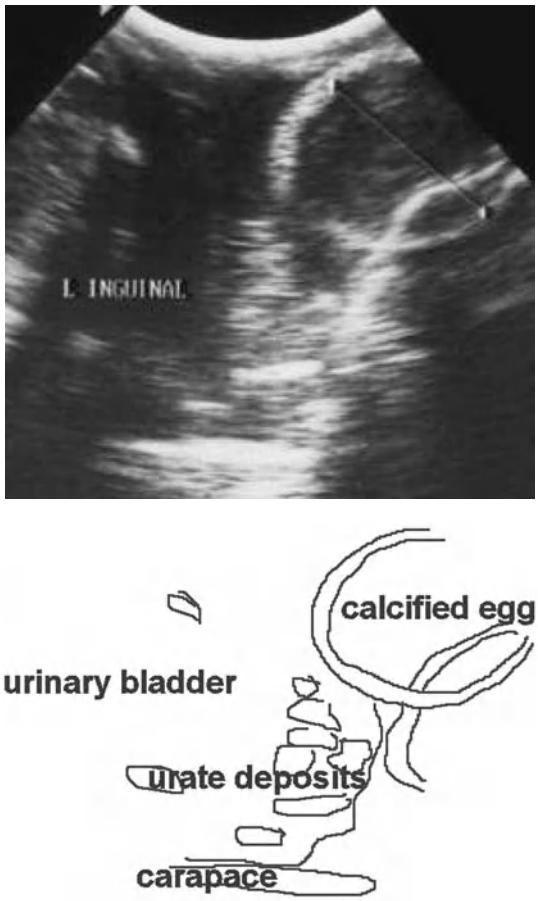
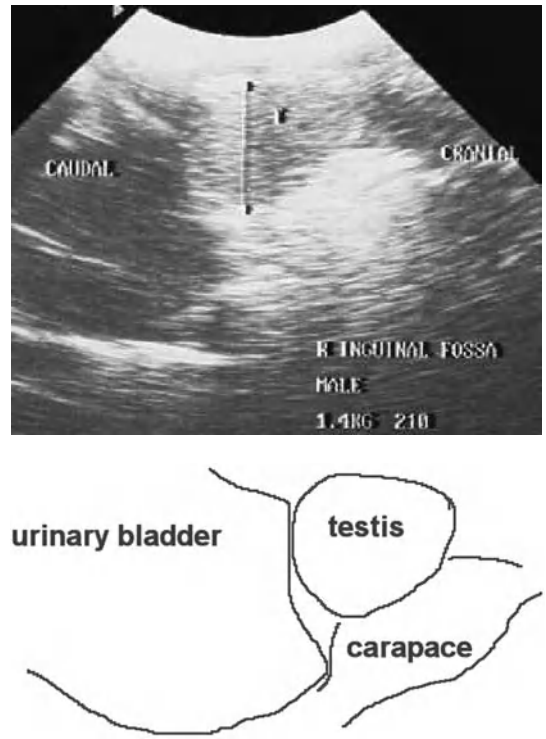


Figs 8.19 & 8.19a Ultrasonographic image of the caudal coelomic cavity in a healthy adult female Testudo sp., as seen from the prefemoral scanning window, showing calcified eggs.

Eggs should be examined for fracture. Any which enter the bladder acquire a rough margin of urate crystals.



Figs 8.20 & 8.20a Ultrasonographic image of a testis in a healthy adult male Testudo hermanni. This animal was examined in summer and had access to females.

The testis is a homogeneous, rounded structure immediately below the kidney. Size fluctuates markedly with season.

as a thin-walled, pleated and fluid-filled tube, curling past the ovaries and bladder.

Testicles are near-round, homogeneous, solid bodies, which lie in a consistent position cranioventral to the kidneys (Figs 8.20–8.20a). They may be larger in males in contact with females in summer. Those of Mediterranean tortoises may reach 20 mm diameter in summer when contact with females is allowed. Frye *et al.* (1988) reported a single case of testicular neoplasia in a desert tortoise *Gopherus agassizii*.

Eggs

It is possible to determine the state of development of the embryo within the egg, at least in the latter stages of incubation, using ultrasonography (Wilkinson: unpublished observations). A water-filled glove is applied to the shell and the transducer applied to the glove. A coupling gel should be avoided as it may block pores and cause suffocation. Infertile eggs contain a uniformly rounded yolk. Viable eggs contain an irregular embryo in which the echogenic developing spine is the most readily discernible feature. In frequently-encountered species, such as *Terrapene* and *Testudo* spp., the embryo is too small for a heartbeat to be visualised.

SUMMARISED INTERPRETATION

Table 8.3 summarises what may be seen on ultrasound scanning of chelonians.

RADIOGRAPHY

(Stephen Hernandez-Divers, Maud Lafortune)

Radiography can usefully be applied to the examination of the chelonian skeletal system, lung and gastrointestinal tract. Calcified eggs and uroliths can be located, although very little information will generally be gained concerning the kidneys, liver, heart, thyroid, pancreas or spleen, unless they are mineralised or grossly enlarged.

There are substantial anatomical differences between chelonians and more domesticated patients. These differences make it difficult to extrapolate the pathology, indications, contraindications, specificity, and sensitivity of diagnostic radiology in tortoises and turtles. Indeed, the use of accepted mammalian techniques may result in completely misleading radiographs (Silverman & Janssen 1996; Jackson & Sainsbury 1992) (Figs. 8.21–8.23).

Various contrast studies show excellent promise. Barium sulphate (30%) can be used for gastrointestinal studies, although the authors prefer water-soluble iodine compounds (e.g. Iohexol, Omnipaque 350, Nycomed or Gastrografin®) for gastrointestinal, urogenital and intravenous techniques. Radio-opaque beads (barium impregnated polyethylene spheres or BIPS) are useful in the assessment of digestive tract function and do not compromise potential surgery. However, they do not provide information on mucosa detail or filling defects.

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Table 8.3 Interpretation of ultrasound scans.

Thyroid	Ovoid organ cranial to heart and major vessels. Up to 14 mm diameter in adult Mediterranean *Testudo* spp. Enlarged in iodine deficiency or neoplasia.
Heart	In midline, adjacent to plastron. Single ventricle separated from two atria by echogenic, moving valves. Gout and granulomata can be detected. Pericardial fluid may be normal. Cytology required for diagnosis of pathological effusion. Atrial fractional shortening 30%–50% across widest point.
Liver	Homogeneous tissue lying across coelom caudal to heart. Focal lesions caused by neoplasia or infection.
Gall bladder	Hypoechoic organ 3–6 mm diameter on right side of liver at caudal margin.
Bladder	Can be difficult to distinguish from coelomic effusion. Intensely echoic, small urate crystals are commonly observed. Pathological presence of eggs has been reported.
Kidneys	Rounded with a broad head tapering to a thinner tail. Homogenous cortex with thin, hypoechoic medulla. Renal gout (echogenic mineralisation) is frequently observed.
Testes	Round and homogeneous. Immediately cranioventral to kidneys. Much larger in summer in males with access to females. Neoplasia has been reported.
Eggs	Incubating eggs may be scanned. The developing embryo is usually apparent if present. The spine is particularly echogenic.
Female reproductive tract	Developing follicles are round and homogeneous. They may be found throughout the coelom. Large numbers (>15) suggest follicular stasis. Up to 25 mm in diameter at ovulation in Mediterranean species. Non-calcified ova of this size may be visible within the oviduct. Shelled eggs are usually obvious. Empty oviduct is visible as a pleated tube.

Fig. 8.21 Normal dorsoventral radiograph of a conscious red eared terrapin (*Trachemys scripta*). Conscious chelonians will often hold all appendages inside the shell making radiographic imaging and interpretation difficult.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

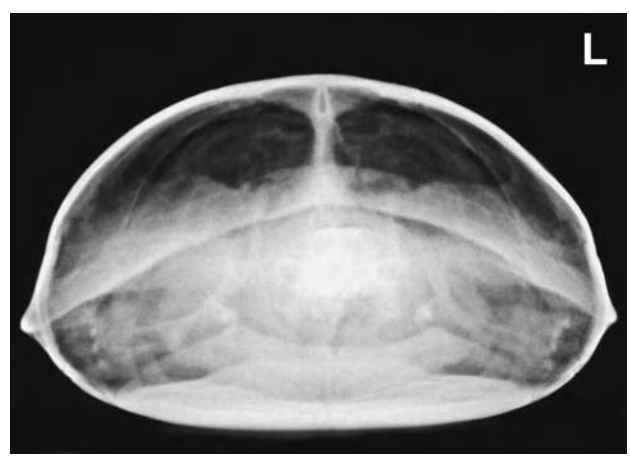

Fig. 8.22 Normal craniocaudal (horizontal beam) radiograph of a conscious red eared terrapin (*Trachemys scripta*). Conscious chelonians will often hold all appendages inside the shell making radiographic imaging and interpretation difficult.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

EQUIPMENT

Radiology units

A radiographic unit capable of producing 300 mA at exposure times of 0.01 sec is to be preferred, but is generally out of the financial range of most practices. Generally speaking, standard units that offer a step-wise increase in kVp sacrifice mA, because as the kVp increases, the mA decreases. There are significant drawbacks with such systems but nevertheless diagnostic radiographs can be obtained.

Higher mA settings permit the use of lower kVp, which is more important when dealing with reptiles that possess less radiopaque skeletal structures compared to mammals. The following recommendations are made:

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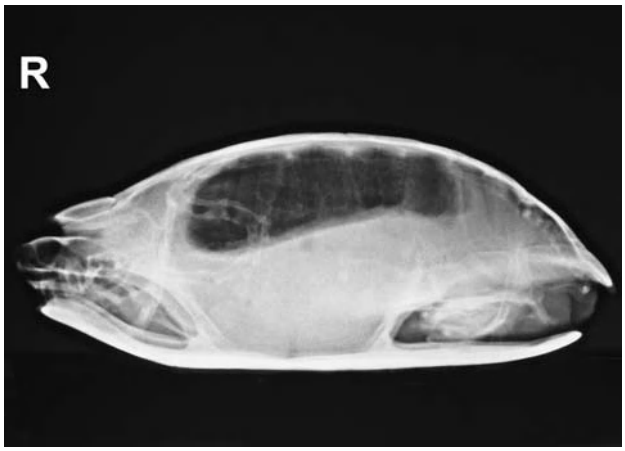

Fig. 8.23 Normal lateral (horizontal beam) radiograph of a conscious red eared terrapin (*Trachemys scripta*). Conscious chelonians will often hold all appendages inside the shell making radiographic imaging and interpretation difficult.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

- 40–60 kVp–small to medium chelonians (<2 kg);
- 60–80 kVp–medium chelonians (2–8 kg);
- 80–100 kVp–large chelonians (>8 kg).

Radiology units often possess two focal-spot sizes (produced by two filaments). The smaller the focal spot, the smaller the electron output but the better the image detail. Fine detail skull or extremity radiographs will benefit from a small focal spot of between 0.3 mm–0.6 mm.

The quality of the radiology unit is obviously important, but secondary to proper implementation and radiographic interpretation. Reptilian radiology requires the use of a horizontal beam, and therefore the tube head must have the capacity for 90° rotation. Horizontal-beam technique also requires some form of cassette holder and careful consideration must be given to health and safety requirements, including barium-painted walls and doors.

Film and intensifying screens

The huge variation in size and weight of chelonians means that a variety of screen and film combinations will be needed. A system capable of taking detailed skeletal radiographs of a 50 g neonate *Testudo graeca* will not be at all useful for taking lung-field radiographs of a 30 kg *Geochelone sulcata*.

For coelomic radiology of smaller specimens or extremity radiology of larger specimens, high detail, rare-earth intensifying screens and compatible film are recommended, with the selected system designed for a kVp range of 40–60 kVp. Where high detail is vital, the use of non-screened film may be used, but the long exposure times needed can require chemical restraint of the patient. The use of the Lanex Fine screen and film produced by Kodak has proven effective, but an equally acceptable system appears to be the use of mammography film-screen combinations. For larger animals, faster films, screens and grids offer comparatively less detail but may be essential to obtain diagnostic images.

RADIOGRAPHIC VIEWS

Dorsoventral (vertical beam)

Sedation or anaesthesia is generally unnecessary. Simple placement of the patient onto the cassette is usually sufficient to obtain a quality radiograph (Figs 8.24–8.25). Ideally, the head and limbs should be extended from the shell, but this is seldom essential in practice unless specifically examining the extremities.

More active animals can be restrained by taping them to the cassette or by placing them in a radiolucent container, e.g. cardboard box, although this should be avoided, particularly with smaller specimens (and lower settings) as material artefacts may appear on the radiographs.

Dorsal recumbency is stressful and may displace the organs, making interpretation difficult.

Applying a piece of tape between the animal's caudal carapace and the cassette so it cannot move forward will often make the animal extend its limbs in an effort to escape. This may allow a radiograph to be taken of a conscious, unrestrained animal with

Fig. 8.24 Positioning for dorsoventral, vertical beam radiograph. Note the presence of a label and left-right marker, which are essential for proper interpretation.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.25 Dorsoventral radiographs of a box tortoise (*Terrapene spp.*). The left image is taken using conventional film and cassettes, while the right image represents a digital radiograph. Note the improved contrast of the digital image.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

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limbs in extension. This view is useful for the detection of eggs and foreign bodies. Mineralisation of the pelvis can be assessed. The lungs are, however, obscured by the superimposition of the coelomic viscera.

Lateral (horizontal beam)

For lateral horizontal beam radiographs the chelonian is placed on a small, central, plastron stand. Lifting the animal clear of the ground will encourage the animal to extend its head and limbs, but the tortoise will remain immobile (Figs 8.26–8.27).

Fig. 8.26 Positioning for lateral, horizontal beam radiograph. The tortoise is elevated (in this case with a small pot) so that the limbs are extended.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

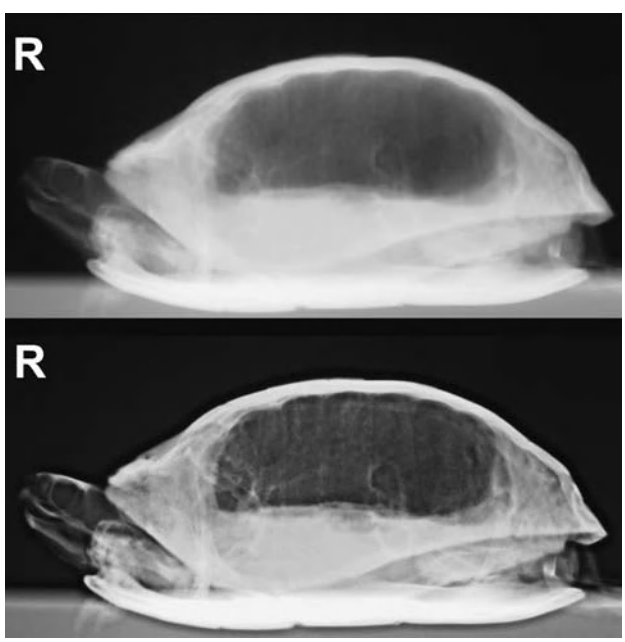

Fig. 8.27 Lateral (horizontal beam) radiographs of a box tortoise (*Terrapene sp.*). The top image is taken using conventional film and cassettes, while the lower image represents a digital radiograph. Note the improved contrast of the digital image.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.28 Positioning for craniocaudal, horizontal beam radiograph. The tortoise is elevated (in this case with a small pot) so that the limbs are extended.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Both left and right lateral projections can be taken, with the lateral edge of the shell touching (or as close as possible to) the cassette.

This view allows localisation in two planes of lesions visible on craniocaudal or dorsoventral views.

Craniocaudal (horizontal beam)

The third basic coelomic view is the horizontal anterior-posterior (or craniocaudal) view. Again, the animal is positioned on a central plastron stand, with the caudal edge of the carapace touching (or as close as possible to) the cassette, with the head facing the X-ray tube and the beam centred on the cranial rim of the carapace (Figs 8.28–8.29).

Tipping the patient may cause abnormal displacement of the internal organs and create radiographic artefacts.

This view has the advantage of isolating the lung fields and allowing the two sides to be compared.

HEAD AND LIMBS

Radiology of the head and limbs requires their exteriorisation from the shell and this will usually require general anaesthesia. The use of sandbags and tape will aid positioning. Standard interpretation requires both true lateral and dorsoventral views.

MUSCULOSKELETAL SYSTEM

The common musculoskeletal anomalies that can be detected on radiographs include metabolic bone diseases (often secondary nutritional hyperparathyroidism), fractures, dislocations, joint diseases, osteomyelitis and, rarely, neoplasia.

Nutritional metabolic bone disease

Nutritional metabolic bone disease (secondary nutritional hyperparathyroidism) produces soft shells, beak and claw abnormalities and classic radiographic changes. Bone opacity is reduced and

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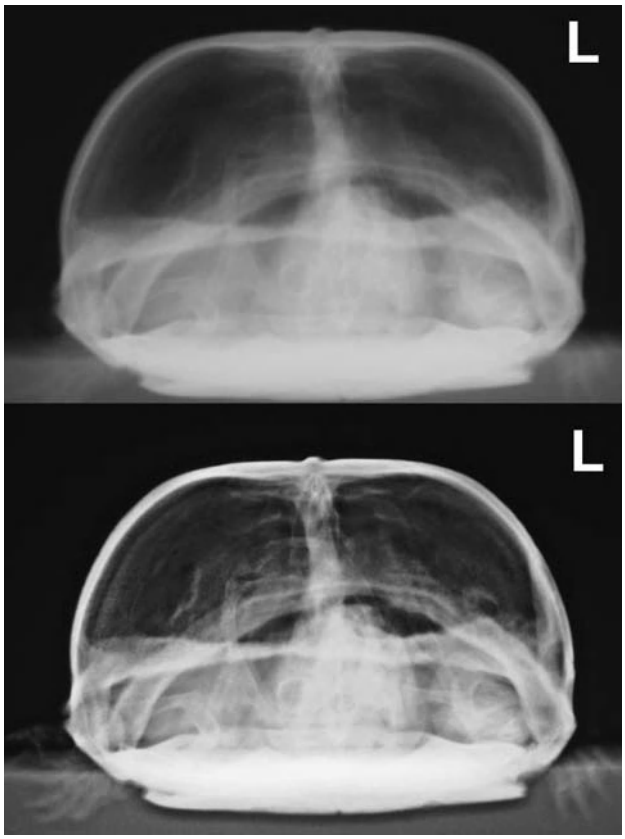

Fig. 8.29 Craniocaudal (horizontal beam) *radiographs of a box tortoise* (Terrapene sp.). The top image is taken using conventional film and cassettes, while the lower image represents a digital radiograph. Note the improved contrast of the digital image.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

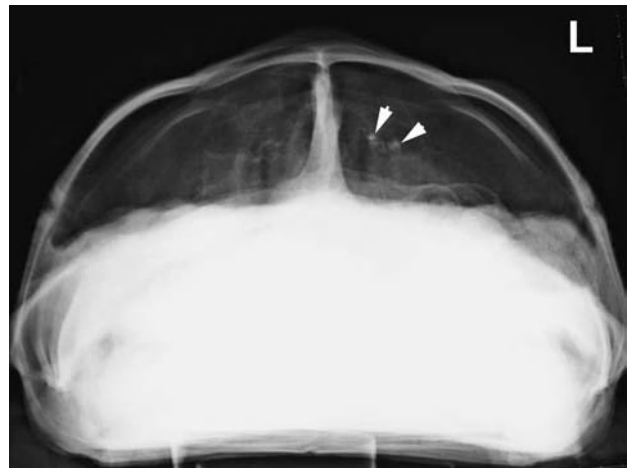

Fig. 8.30 Craniocaudal (horizontal beam) radiograph of an African spurred tortoise (*Geochelone sulcata*). Note the increased mineralisation of the pulmonary vessels secondary to secondary renal hyperparathyroidism.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

there is often obvious cortical thinning. Commonly, shell (and less commonly limb) thickening occurs as a result of periosteal fibrous proliferation. The opacity of the pelvic and pectoral girdles provides the best assessment of ossification and mineral depletion (Figs 8.30–8.32b).

Soft-tissue mineralisation

Soft-tissue mineralisation, mineralised gout, or pseudogout may be seen as areas of increased opacity in the periosteal or periarticular areas, or within any visceral tissue.

Fractures

Limb fractures are not particularly common in chelonians, and their diagnosis is usually straightforward. Fractures of the shell may be more subtle and difficult to appreciate, but are usually a consequence of severe trauma (e.g. falls, road traffic accidents, lawnmowers or predators). The situation is further complicated by the lack of periosteal new bone growth and obvious callus formation, so that a radiolucent line may persist in a clinically normal individual with acquired fibrous fracture stabilisation (Figs 8.33–8.39).

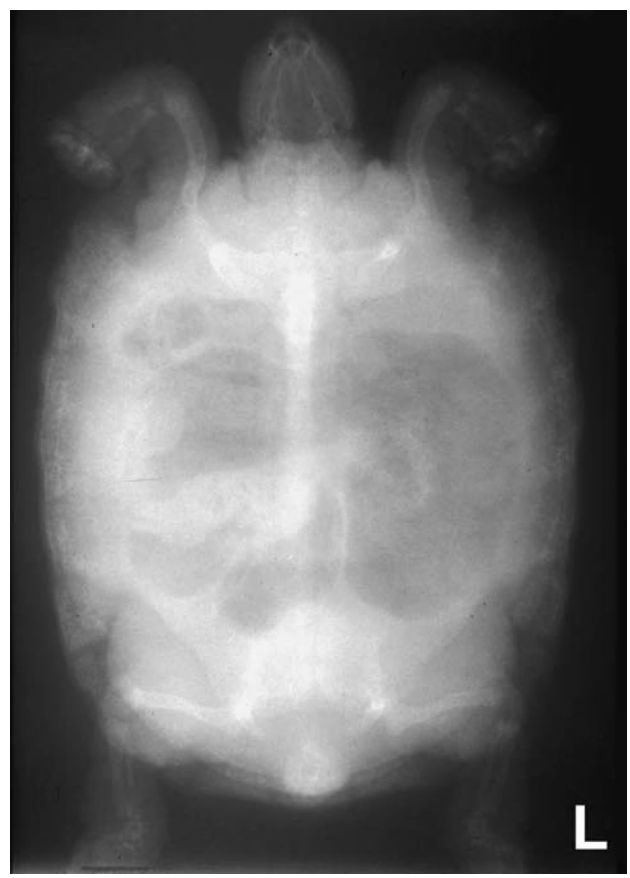

Fig. 8.31 Dorsoventral radiograph of a juvenile spur-thighed tortoise (*Testudo graeca*). Note the generalized demineralisation consistent with secondary nutritional hyperparathyroidism, and the increased intestinal gas due to ileus caused by hypocalcaemia.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

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Fig. 8.32a Dorsoventral radiograph of a juvenile snapping turtle (*Chelydra serpentina*) with generalized poor skeletal mineralisation. History, clinical and radiographic signs were consistent with secondary nutritional hyperparathyroidism.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

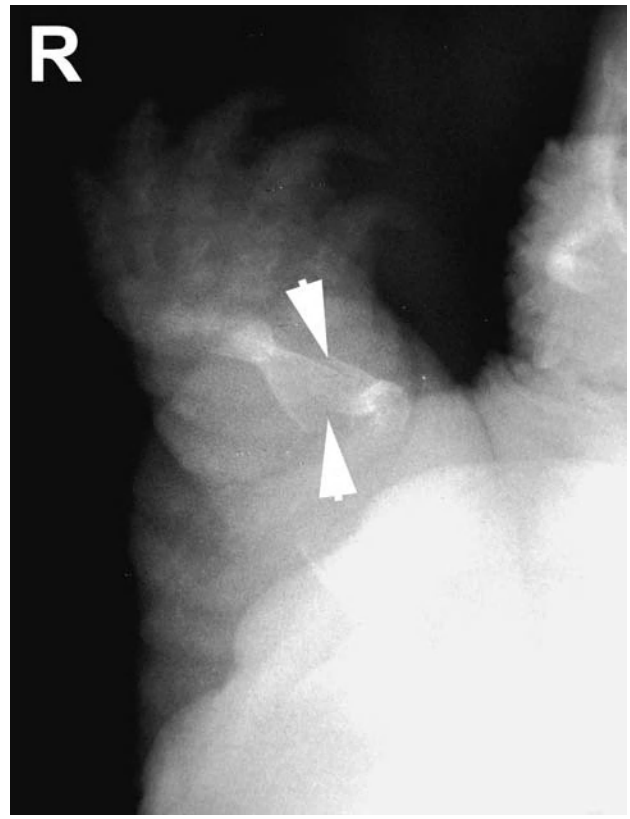

Fig. 8.32b Lateral radiograph of the right forelimb of the same snapping turtle (*Chelydra serpentina*) with secondary nutritional hyperparathyroidism. Note the pathological fractures of the right ulna (arrows).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

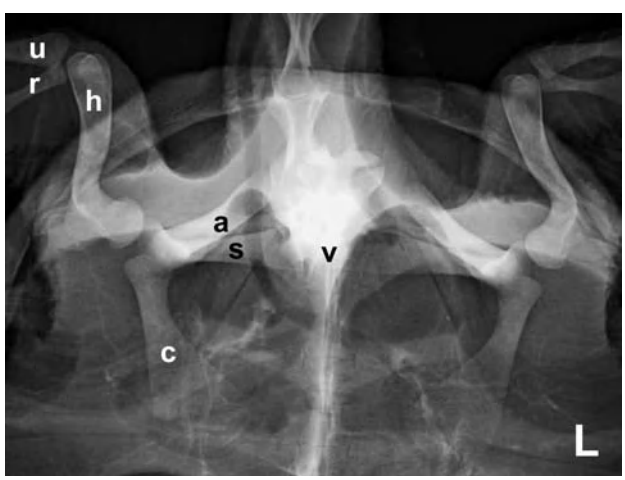

Fig. 8.33 Dorsoventral radiograph of the normal pectoral region of a chelonian.
u—ulna
r—radius
h—humerus
a—acromion process
s—scapula
c—coracoid
v—first (or second depending upon species) fixed vertebra.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Following diagnosis and any fracture repair, follow-up radiographs should be taken at 10 and 20 weeks to assess the repair process, although complete healing and remodelling is likely to take 6–18 months.

Traumatic joint dislocations

On rare occasions, traumatic joint dislocations can also occur. Dislocations of the stifle and elbow are easily appreciated but coxofemoral and scapulohumeral dislocations may be more difficult to detect without lateral, dorsoventral and craniocaudal views. Radiographs taken after 6, 12 and 24 months may follow up restorative joint surgery for evidence of degenerative joint disease or infection (Fig. 8.40).

Degenerative joint disease

Chelonians are amongst the longest lived of animals, and age-related orthopaedic disease, namely degenerative joint disease, is becoming increasingly common. The increase in the prevalence of arthritis in companion tortoises may be related to overfeeding and obesity, confinement and underactivity. Other causes of degenerative joint disease include articular gout, pseudogout and trauma. Evidence of bone lysis at the articular surfaces and joint

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Fig. 8.34a Dorsoventral radiograph of a snapping turtle (*Chelydra serpentina*) that was hit by a car.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.34b Dorsoventral radiograph of the skull of the same animal. Note the left mandibular and maxillary fracture (arrow).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

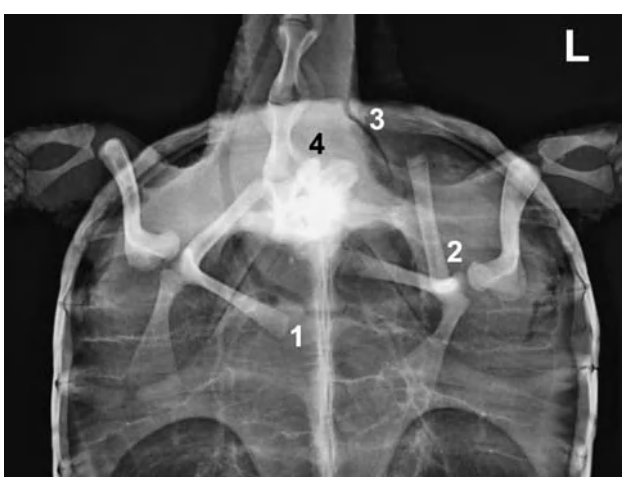

Fig. 8.34c Dorsoventral radiograph of the pectoral girdle region of the same animal. Note (1) the dislocation of the scapular-vertebral junction and subsequent caudolateral rotation of the right pectoral girdle; (2) the scapular fracture resulting in over-riding and an increase in focal bone density; (3) the fracture through the cranial rim of the carapace; (4) and the cervical dislocation.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

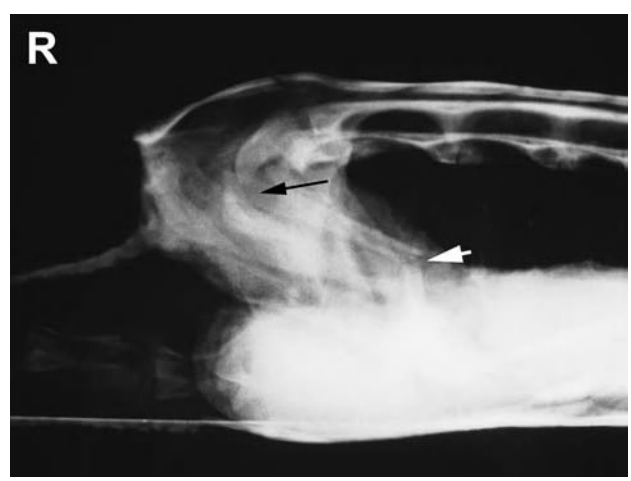

Fig. 8.34d Lateral (horizontal beam) radiograph of the pectoral region of a snapping turtle (*Chelydra serpentina*) that was hit by a car. Note the fracture of the scapula (white arrow) resulting in cranial displacement of the scapula (black arrow).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

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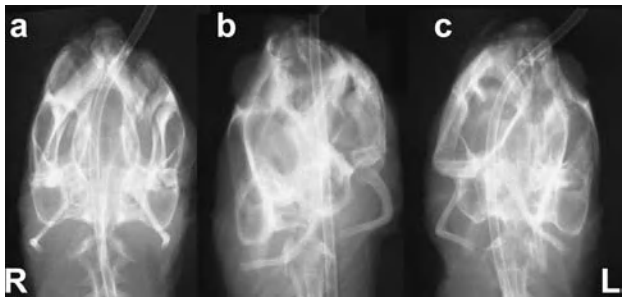

Fig. 8.35 Radiographs of the skull of a red-eared terrapin (*Trachemys scripta*) with a left mandibular and maxillary fracture: a) dorsoventral view; b) left lateral oblique; c) right lateral oblique.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

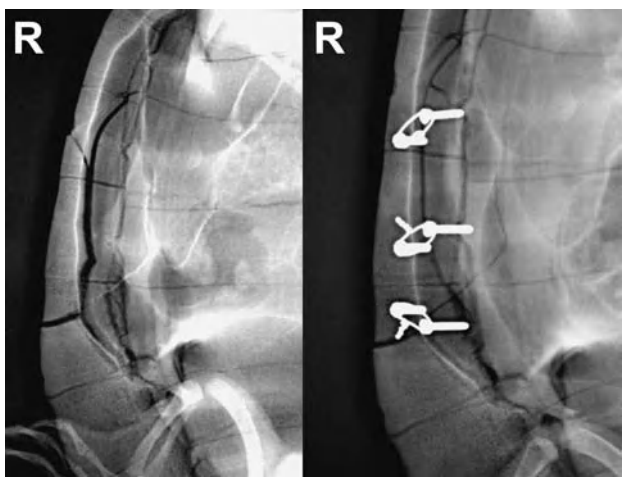

Fig. 8.36a Dorsoventral radiographs of a wild, red-eared slider (*Trachemys scripta*) that was hit by a car. The left radiograph illustrates a displaced fracture of the right lateral plastrocarapacial junction. The right radiograph shows the same region following orthopaedic repair using screws and wire.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

swelling are most noticeable, but careful consideration must be given to differentiating between septic and aseptic arthritis, as both may appear similar radiographically.

Osteomyelitis

Osteomyelitis is a common presentation in reptiles and tends to be lytic rather than proliferative as it is in mammals. There is often gross enlargement and distortion of the local anatomy with loss of corticomedullary definition. Following successful treatment, usually surgical, a lytic area will often remain but may not be indicative of infection recurrence or sequestrum formation. Septic arthritis is usually represented by gross enlargement of the joint, increase in soft tissue density within the joint space and lysis of the articular bone surfaces.

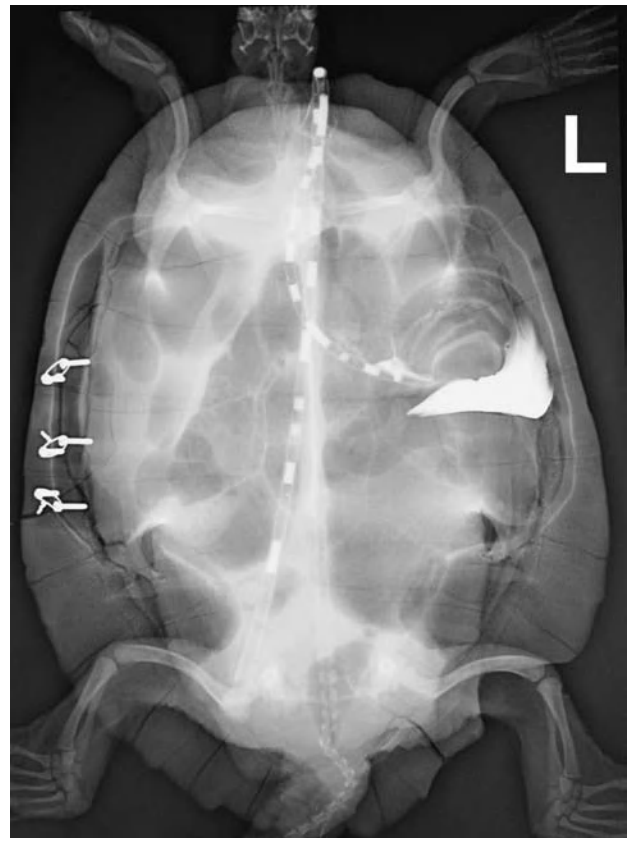

Fig. 8.36b Same animal as in 8.36a. An oesophagostomy tube has been placed to facilitate oral dosing and nutritional support. A small amount of barium has been injected down the tube to verify correct placement in the stomach.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

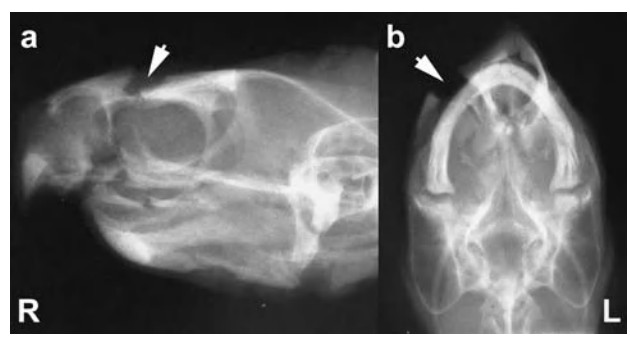

Fig. 8.37 Lateral (a) and dorsoventral (b) radiographs of an Afghan tortoise (*Testudo horsfieldi*) with a displaced fracture (arrows) of the rostral maxilla.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

GASTROINTESTINAL SYSTEM

The chelonian gastrointestinal tract consists of the buccal cavity, oesophagus, stomach, small and large intestine and cloaca. The radiographic appearance of the tract will depend upon the temperature and nutritional status of the animal, the nature of its ingesta and the period of time elapsed between feeding and

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Fig. 8.38a Craniocaudal radiograph of the right forelimb of an eastern box tortoise (*Terrapene carolina*) with a displaced fracture of the humerus.
(Courtesy of Sonia Hernandez-Divers and the Radiology Department, Cornell University)

Fig. 8.38b Lateral radiographs of the pelvic limbs of the same eastern box tortoise (*Terrapene carolina*). Note the incidental and unexplained absence of the right metatarsal bones.
(Courtesy of Sonia Hernandez-Divers and the Radiology Department, Cornell University)

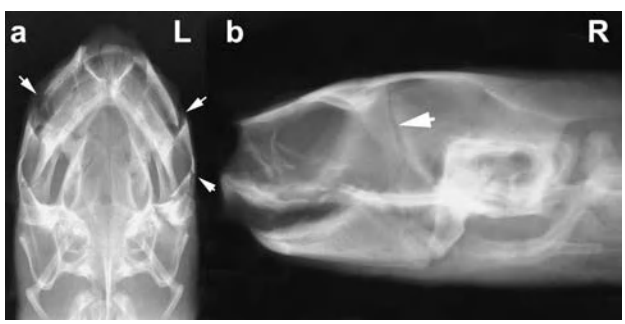

Fig. 8.39 Dorsoventral (a) and lateral (b) radiographs of a Hermann's tortoise (*Testudo hermanni*) that sustained head trauma following a fall. Note the multiple fractures (arrows).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

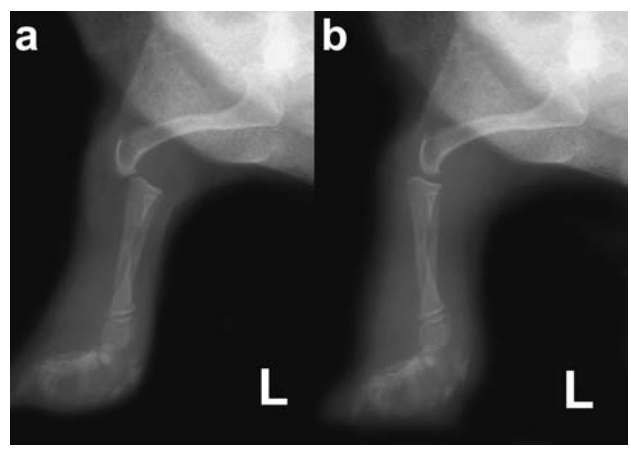

Fig. 8.40 (**a**) Lateral radiograph of the left hind limb of an adult spur-thighed tortoise (*Testudo graeca*) demonstrating luxation of the stifle joint. (**b**) Post-operative radiograph following cruciate reconstruction and lateral joint imbrication.
(Reprinted from Hernandez-Divers, S. J. (2002). Diagnosis and surgical repair of stifle luxation in a spur-thighed tortoise (*Testudo graeca*). Journal of Zoo and Wildlife Medicine **33**:125–130.)

radiographic examination. The chelonian alimentary tract is shorter than that of mammals but the transit time is also slower. The gastrointestinal tract is best appreciated using a dorsoventral radiograph. The pancreas and liver are not usually discernible, although positive contrast in the stomach can be used to indicate the caudal border of the liver.

Contrast studies

The use of gastrointestinal contrast studies can help to differentiate between digestive- and non-digestive-tract diseases. These techniques can also help to distinguish between intraluminal diseases (e.g. radiolucent foreign bodies) and intestinal diseases (e.g. abscess or neoplasia), and aid in the diagnosis of gastrointestinal perforation.

Metoclopramide (0.5 mg/kg IM) and other gastrointestinal modifiers may reduce total transit time; in any case, their use is contraindicated in cases of complete blockage and their efficacy in chelonians unproven. Tothill *et al.* (1999) could not show any statistically significant effect on total gastrointestinal transit times when prokinetik drugs (cisapride, metoclopramide and erythromycin) were used in the desert tortoise (*Gopherus agassizii*).

Similar techniques and media can be used for retrograde, large-intestinal studies, the media being introduced into the large intestine via a lubricated round-tipped catheter. The same volumes can be employed but administration should stop as soon as any resistance is noted. Occasionally, contrast media may enter the bladder or ureters and it is preferable to evacuate the colon afterwards, especially when using 30% barium sulphate, although most chelonians will do this automatically when placed into shallow, warm water.

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Barium sulphate

The use of 30% barium sulphate in two Greek tortoises (*Testudo graeca*) has demonstrated that gastric emptying can take 23–80 hours, with a total transit time of 25–28 days (Holt 1978). More recently, the use of water-soluble iodine contrast media has been shown to be superior to barium sulphate as these media offer shorter transit times but reduced mucosal detail (Meyer 1998).

Iodinated media

The iodine salt amidotrizoate sodium and amidotrizoate meglumine based (370 mg iodine/ml) water-soluble Gastrografin® (Schering) has rapid gastrointestinal transit times and is the contrast medium of choice if perforation of the gastrointestinal tract is suspected or if surgical intervention may be necessary.

Following oral administration of 1 ml/130 g body weight, serial radiographs are taken at 0, 20, 40, 60, 120 and 240 minutes at 30°C. Further radiographs can be taken if transit time is prolonged. The transit time is temperature dependent with mean total transit times for 31°C, 21°C and 15°C being 1.5–4.0 hours, 3.0–8.0 hours and 8.0–24.0 hours respectively (Meyer 1998). Patients should be well hydrated when using these substances.

Barium impregnated polyethylene spheres (BIPS)

Radio-opaque beads are also useful diagnostic tools if surgery is anticipated.

Alimentary blockage

Alimentary blockage may be caused by intraluminal, intestinal or extraluminal disease. Intraluminal obstruction can be caused by helminth parasites, ingested foreign bodies, intraluminal neoplasia or abscessation (Rahal *et al.* 1998). Radiographically, enlargement of the intestinal diameter with an accumulation of radio-opaque material is characteristic. Gas patterns are not always obvious or necessarily even present (Figs 8.41–8.47).

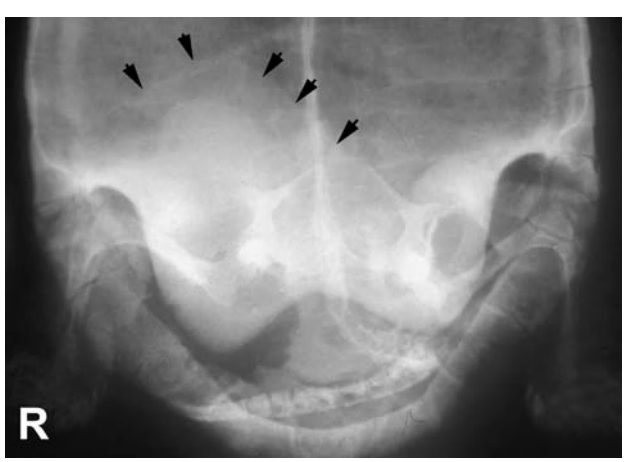

Fig. 8.41 Dorsoventral radiograph of an adult spur-thighed tortoise (*Testudo graeca*). The arrows demark a large coelomic abscess.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.42a Dorsoventral radiograph of a red-footed tortoise (*Geochelone carbonaria*) with a gas-distended loop of small intestine due to intussusception.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

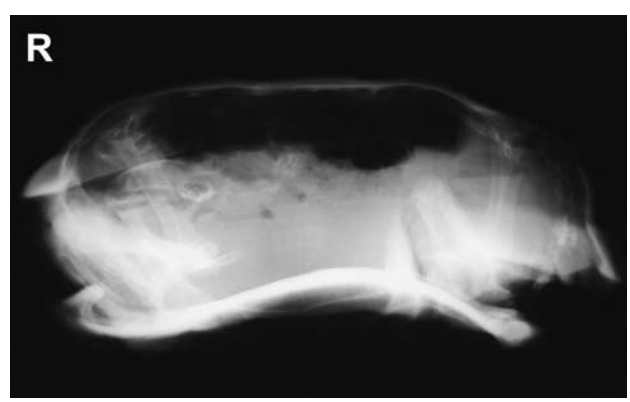

Fig. 8.42b Dorsoventral radiograph of the same red-footed tortoise (*Geochelone carbonaria*) with small-intestinal intussusception.
Note that the radiographic signs are not readily apparent on this view. This exemplifies the need for multiple views in chelonian radiology.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Lead poisoning

Lead poisoning is uncommon in chelonians (Borkowski 1997). Radiographic demonstration of metallic material within the gastrointestinal tract, along with clinical signs and serological evidence of lead or zinc intoxication, are diagnostic.

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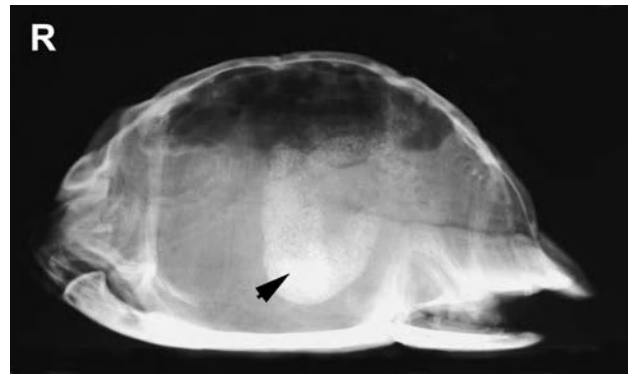

Fig. 8.43a Dorsoventral radiograph of a captive box tortoise (*Terrapene carolina*) with large-intestinal constipation and obstruction.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.43b Lateral (horizontal beam) radiograph of the same tortoise as in Fig. 8.43a.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

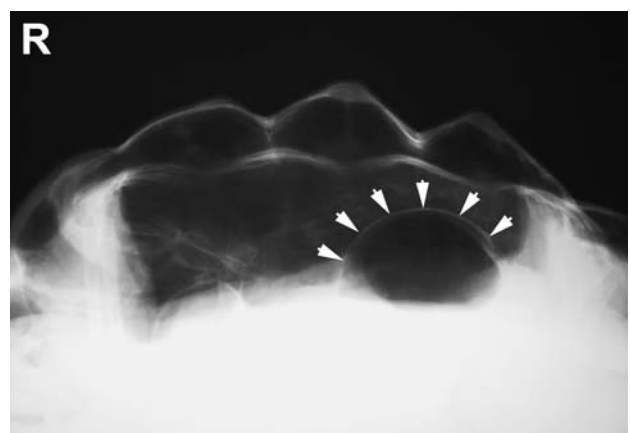

Fig. 8.44a Dorsoventral radiograph of a leopard tortoise (*Geochelone pardalis*) with severe large-intestinal obstructive ileus. An oesophagostomy tube is also present, terminating within the compressed stomach.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.44b Lateral (horizontal beam) radiograph of the same leopard tortoise (*Geochelone pardalis*) with obstructive ileus. A gas-distended loop of large intestine is visible (arrows).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

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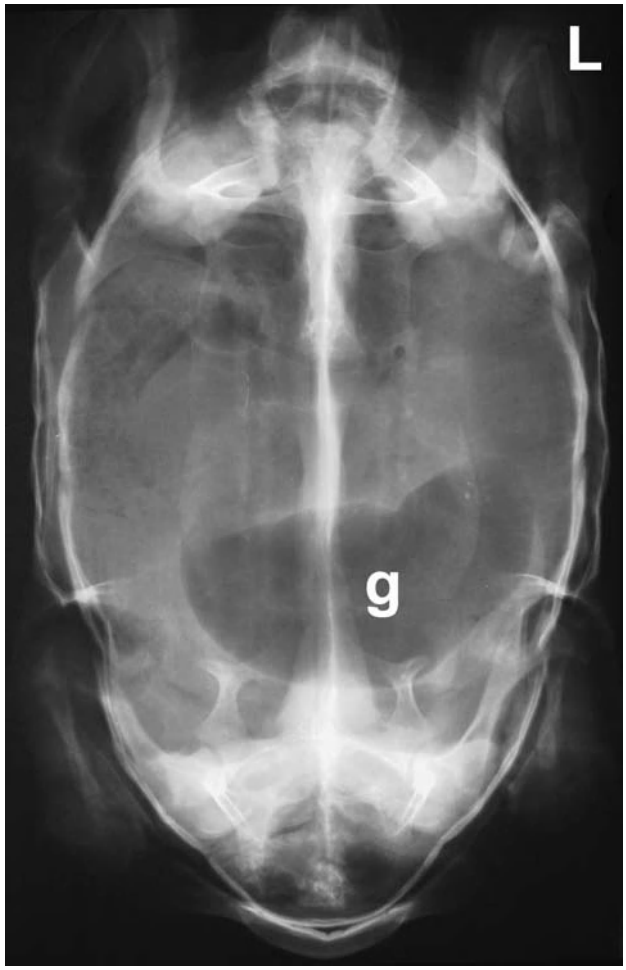

Fig. 8.45 Dorsoventral radiograph of a leopard tortoise (*Geochelone pardalis*) with severe large intestinal ileus secondary to bacterial colitis; note the gaseous distension of the intestinal viscus (g).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.46 Dorsoventral radiograph of an adult marginated tortoise (*Testudo marginata*) with pneumo-oesophagus (arrows).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

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Fig. 8.47 Dorsoventral radiograph of an African spurred tortoise (*Geochelone sulcata*) with a large coelomic abscess and coelomitis. Note that both bronchi have been displaced to the left (arrows) and that the coelomic abscess is so large that even the lung fields (Lu) have been severely compressed.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

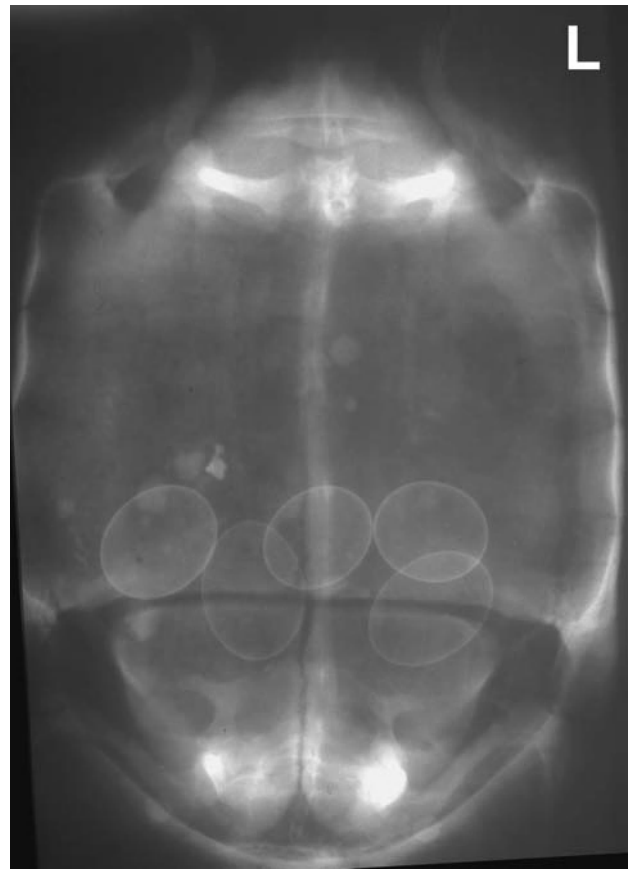

Fig. 8.48 Dorsoventral radiograph of a normal gravid spur-thighed tortoise (*Testudo graeca*).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

UROGENITAL SYSTEM

Assessment of reproductive dysfunction is probably the most common reason for chelonian radiology.

Genital tract

Ovaries and testes

The chelonian ovary is not radiographically visible, but follicular stasis may be inferred from a persistent increase in mid-coelomic soft-tissue presence. Ovarian necrosis and yolk coelomitis will often result in a severe coelomitis and generalised loss of detail.

Testes are adherent to or even embedded within the structure of the kidney and are not usually appreciable.

Egg

The chelonian egg has a calcified shell, which is readily identifiable on plain dorsoventral radiographs. Normal eggs are characterised by radiolucent centres and thin, radio-opaque, evenly-calcified shells. The radiographic size of the oval egg will vary, depending upon orientation, but significantly larger eggs will be obvious (Figs 8.48–8.49).

Fractured eggs have a folded appearance and have lost the smooth appearance of their whole counterparts. It is important that all potentially dystocic chelonians are radiographed, as the medical induction of oviposition using oxytocin may be contraindicated in the presence of large, abnormal or fractured eggs.

Eggs that are not laid normally may remain within the shell glands for prolonged periods of time, which results in thick, but usually even, shells.

On occasion, eggs may remain within the coelomic cavity but outside the oviducts. To determine radiographically that an egg is free-floating within the coelomic cavity is next to impossible. The only possible radiographic clue may be an unusually cranial or lateral position of the egg.

Eggs can also pass into the bladder. This appears to be most common when prior induction with oxytocin has failed. The eggs are forcibly expelled from the shell gland into the urodeum whence they then fall through the wide urethra into the bladder. Initially, it may be impossible to determine whether eggs are within the oviduct or the bladder. However, with time, urate deposits accumulate on the eggs, giving them a roughened appearance. In some cases the tortoise may not present any clinical signs until the following year, when a radiograph will demonstrate normal eggs within the oviducts and thickened, roughened eggs within the bladder.

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Fig. 8.49 Dorsoventral radiograph of an adult female spur-thighed tortoise (*Testudo graeca*) with a normal egg and abnormal large egg. (Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Urinary tract

Bladder

The bladder is difficult to evaluate radiographically because it is either small and empty or large and full of fluid. Normal urates are of course radiolucent, so their presence is no help. In cases of cystic calculi, concrete urate accumulations become dense, assisting their visualisation. Care must be taken not to be fooled by the occasional cranial position of a bladder stone, as the chelonian bladder has an extremely large capacity and can extend far cranially.

Pneumocystography can be done by catheterising the urethra and flushing the bladder contents before injecting air at 10–20 ml/kg, taking care to stop if premature resistance is encountered. Such techniques can sometimes help differentiate between eggs within the bladder and those within the oviducts.

The addition of 2–5 ml of water-soluble contrast medium (iohexol) into the air-filled bladder produces a double-contrast pneumocystogram. Following the procedure, air and contrast media should be evacuated from the bladder.

Kidneys

Kidneys are retro-coelomic, located in the dorsocaudal region and are difficult, if not impossible, to identify on plain radiographs. Pathological conditions such as nephrocalcinosis can delineate the renal parenchyma.

Intravenous urography permits a better assessment of both the kidneys and the ureters. The patient should be starved for 48 hours (to reduce the quantity of ingesta within the alimentary tract and aid kidney visualisation) and an intravenous catheter placed into the right jugular vein. Using a contrast medium suitable for mammalian urography (e.g. Iohexol, Omnipaque 300, Nycomed), 300–500 mg/kg iodine is injected as a bolus via the catheter, with serial dorsoventral and lateral radiographs taken at 0, 0.5, 1, 5, 10, 20 and 60 minutes at 30°C.

CARDIOPULMONARY SYSTEM

Heart

The heart is located within a pericardial sac in the cranial third of the coelomic cavity, resting above the plastron, but is not radiographically discernible.

Lungs

Radiography can provide a very good assessment of pulmonary disease, as air provides excellent contrast despite the presence of the carapace. Lateral and craniocaudal views are most informative, although severe consolidation can be appreciated on a dorsoventral image. It is important to take both left and right lateral radiographs, as the greatest detail will be of the lung closest to the cassette. In addition, the craniocaudal view is vital to compare and contrast the left and right lungs. Using both lateral and craniocaudal views, it is possible to pinpoint focal lesions for microbiological sampling or intrapneumonic therapy (via carapacial drilling) (Divers 1998b). Repeat radiographs can help to ensure the correct position of intrapneumonic catheters (Figs 8.51a–51b).

It is important to account for the position of the limbs, as lung volume will be greatly reduced by the head and limbs being withdrawn into the shell. Likewise, the best lung radiographs will be obtained with the head and limbs extended, although this often necessitates sedation or general anaesthesia.

The chelonian lung is very different from that of mammals. It is comprised of large edicular air spaces interspersed with bands of muscular and connective tissue, giving the overall appearance of a honeycomb with a central cavity. Consequently, the usual alveolar, interstitial, bronchial and pleural patterns seen in mammalian thoracic radiographs do not figure in diagnosis of the chelonian patient.

Lung consolidation due to pneumonia is observed as an increase in lung opacity and may be focal or diffuse, unilateral or bilateral. In cases of diffuse but unilateral pneumonia, dorsoventral radiology can be used to ensure that a lavage catheter (containing a metal stylet) has been introduced into the correct lung. Radiographs can also be used to monitor response to treatment and resolution of the pneumonia.

SUMMARISED INTERPRETATION

Table 8.4 summarises the interpretation of radiographs of the chelonian patient.

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Fig. 8.50a Dorsoventral radiograph of a loggerhead sea turtle (*Caretta caretta*) illustrating the large lung fields and paired pulmonary vessels. (Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

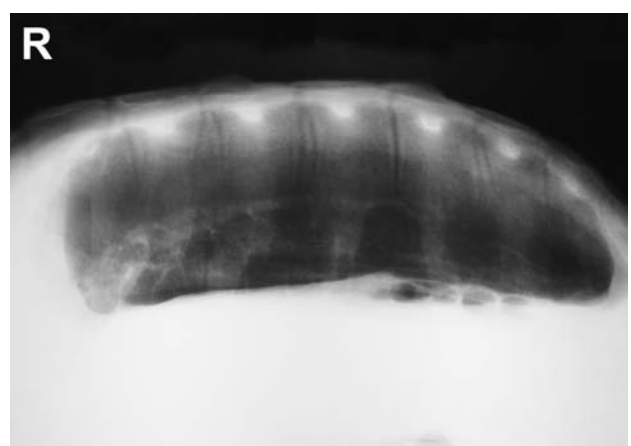

Fig. 8.50b Lateral (horizontal beam) radiograph of a loggerhead sea turtle (*Caretta caretta*) illustrating the large lung fields and prominent pulmonary vessels.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

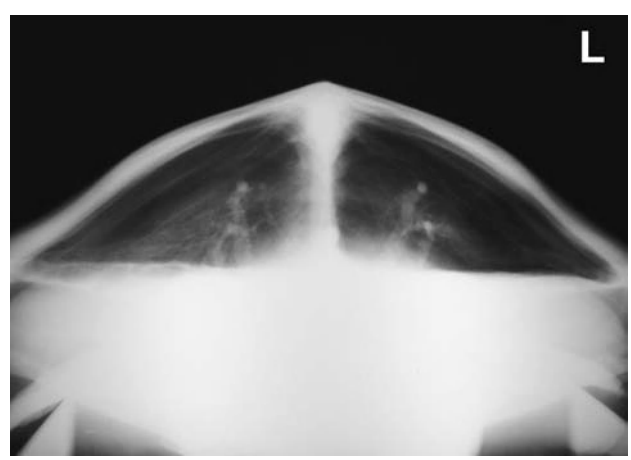

Fig. 8.50c Craniocaudal (horizontal beam) radiograph of a loggerhead sea turtle (*Caretta caretta*) illustrating the large lung fields and prominent pulmonary vessels.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

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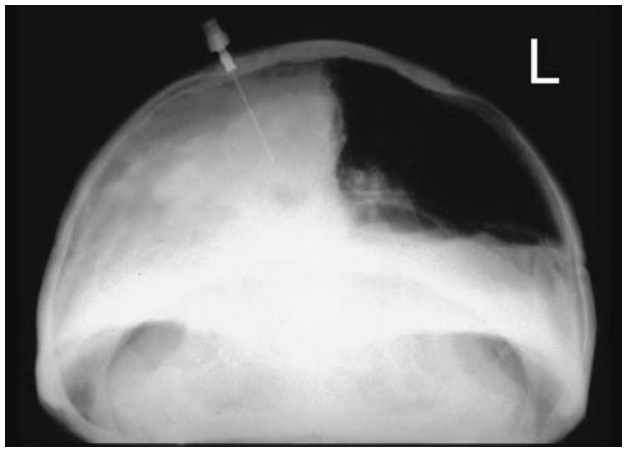

Fig. 8.51a Craniocaudal (horizontal beam) radiograph of a spur-thighed tortoise (*Testudo graeca*) with unilateral pneumonia due to Candida albicans. Note the almost complete consolidation of the right lung field, and the placement of an intrapulmonary catheter through which medication was injected.
(Reprinted from Hernandez-Divers, S.J. (2001). Pulmonary candidiasis caused by Candida albicans in a Greek tortoise (*Testudo graeca*). Journal of Zoo and Wildlife Medicine **32**:352–359).

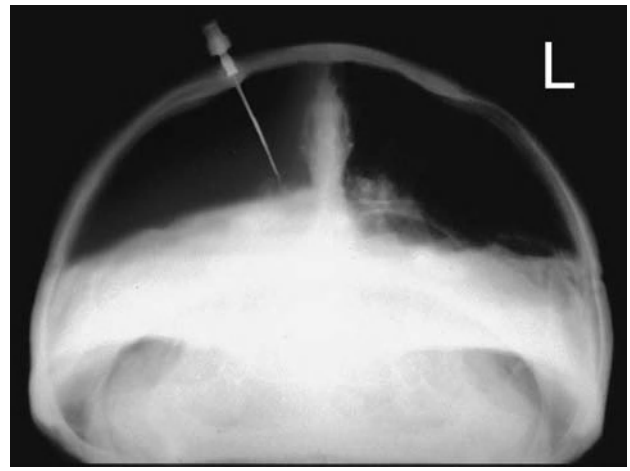

Fig. 8.51b Craniocaudal (horizontal beam) radiograph of the same spur-thighed tortoise (*Testudo graeca*) following 34 days of intrapulmonary amphotericin B therapy. Note the complete resolution of the consolidation, and the dorsal displacement of the post-pulmonary septum (septum horizontale) due to pulmonary fibrosis.
(Reprinted from Hernandez-Divers, S.J. (2001). Pulmonary candidiasis caused by Candida albicans in a Greek tortoise (*Testudo graeca*). Journal of Zoo and Wildlife Medicine **32**:352–359).

Table 8.4 Summary of interpretation of chelonian radiographs.

Musculoskeletal system	Metabolic bone disease	Common. Thin cortices and lucent bones. Assess pelvis and pectoral girdle.
	Soft-tissue calcification	Over-supplementation or dystrophic calcification in inflammatory lesions.
	Fractures (Fig. 8.52)	Uncommon, apart from carapacial trauma and distal limbs.
	Articular gout	Less common. Urate crystals are radiolucent and only radio-opaque when mineralised.
	Septic arthritis	Quite common. Lysis is the dominant feature rather than sclerosis. Soft-tissue swelling. Shoulders and knees are often affected.
Gastrointestinal system (Figs 8.53–8.58)	Endoparasites	Filling defects are sometimes apparent on contrast studies.
	Obstruction	Common, particularly when housed on inappropriate substrate. Often a symptomatic animal with vast amounts of mineral present in distended loops. Contrast studies if in doubt.
	Foreign bodies	Very common. Foreign bodies (particularly gastric) are common in healthy chelonians, therefore their presence in a sick one does not necessarily imply a pathogenic role.
Urogenital system	Follicular stasis	Increased area of soft tissue density in mid-coelom.
	Calcified eggs	The presence of shelled eggs is not necessarily an indication for intervention. Fractured eggs may be a cause of, or a sequel to, dystocia. Abnormally positioned eggs may have escaped into the coelom. Eggs in the urinary bladder acquire a rough margin of urates over time.
	Uroliths	The bladder is very big: uroliths may be unexpectedly cranial or lateral.
	Renal masses	May project into the lung fields.
Respiratory system	Pneumonia	Unilateral or bilateral. Chronic lesions are often discrete. Acute pneumonia is associated with patchy increase in density, usually concentrated ventrally. Radiographs can be used to pinpoint lesions for osteotomy and intrapneumonic therapy.

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Fig. 8.52 Lateral (horizontal beam) radiograph of an African hingeback tortoise (*Kinixys belliana*). Note the normal carapacial hinge (arrow), which should not be confused with a fracture.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.53a Dorsoventral radiograph of a red-eared terrapin (*Trachemys scripta*) with an oesophageal fisherman's hook foreign body and severe gaseous distension of the gastrointestinal tract due to a paralytic ileus.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.55 Lateral (horizontal beam) radiographs of the same animal as in Fig. 8.54 with head and neck a) retracted and b) extended.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

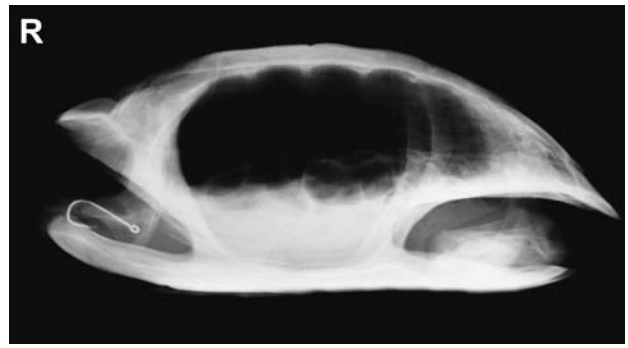
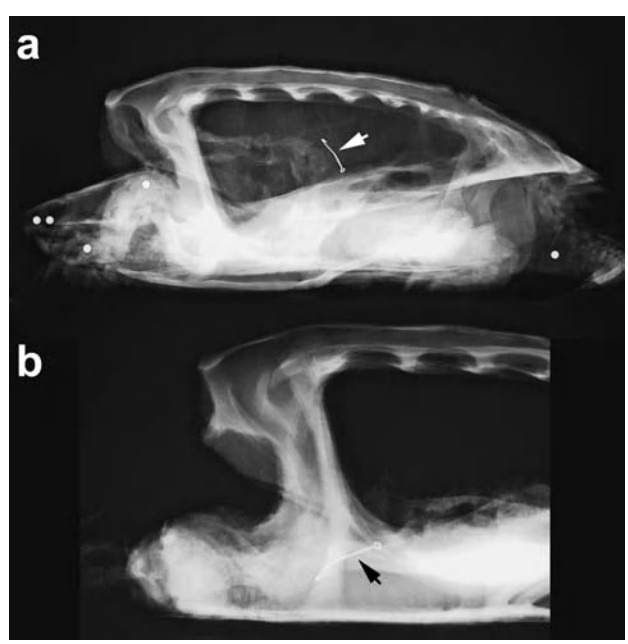


Fig. 8.53b Lateral (horizontal beam) radiograph of a red-eared terrapin (*Trachemys scripta*) with an oesophageal fisherman's hook foreign body and severe gaseous distension of the gastro-intestinal tract due to a paralytic ileus.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

Fig. 8.54 Dorsoventral radiographs of a wild snapping turtle (*Chelydra serpentina*). The spherical radio-dense objects in the head are gun shot: **(a)** a fisherman's hook foreign body is within the mid-coelom. However, by extending the neck **(b)** the hook can be moved to the cranial coelom, confirming that the foreign body is located within the oesophagus, and making surgical removal simpler.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)



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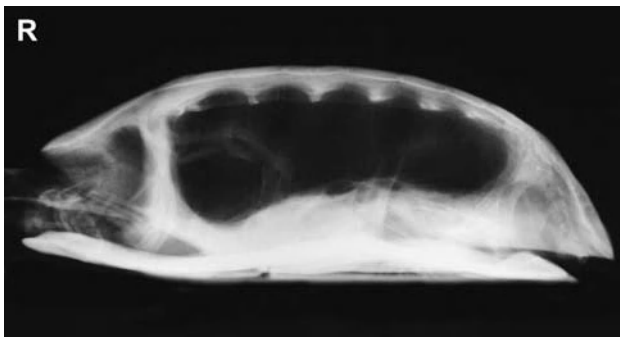

Fig. 8.56 Lateral (horizontal beam) radiograph of an emaciated red-footed tortoise (*Geochelone carbonaria*). Note the reduction in the volume of the coelomic viscera.
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

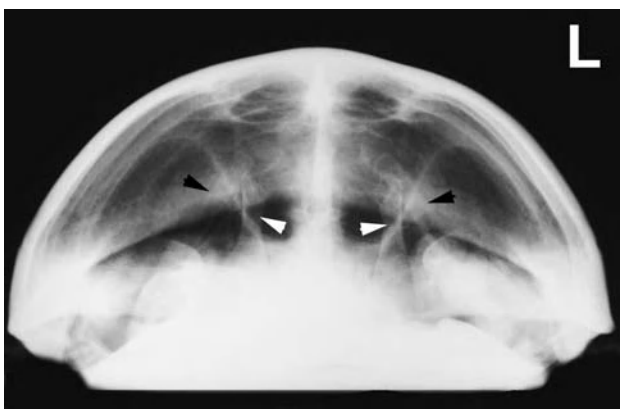

Fig. 8.57 Craniocaudal (horizontal beam) radiograph of an emaciated red-footed tortoise (*Geochelone carbonaria*). Note the pectoral scapulae (black arrows) and the pelvic ilea (white arrows).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

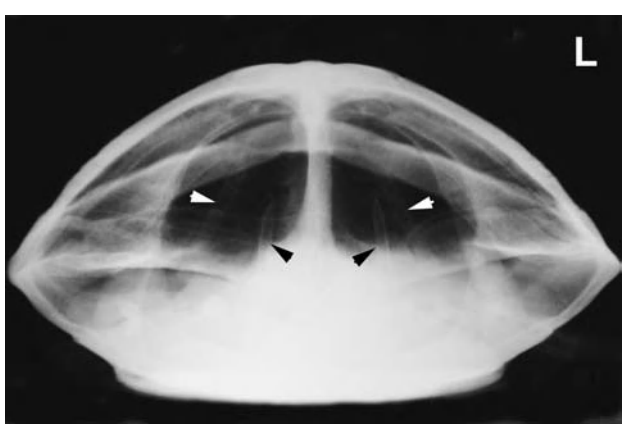

Fig. 8.58 Craniocaudal (horizontal beam) radiograph of a red-eared slider (*Trachemys scripta*). Note the pectoral scapulae (white arrows) and the pelvic ilea (black arrows).
(Courtesy of Stephen J. Hernandez-Divers and Department of Anatomy & Radiology, University of Georgia)

ENDOSCOPY

(Stephen Hernandez-Divers, Maud Lafortune)

Endoscopy has proven to be a most useful diagnostic tool in veterinary medicine. In the field of zoological medicine, the application of diagnostic endoscopy has shown great promise in a variety of species but has probably been most exploited by avian veterinarians, because of their air-sac system (Brearley *et al.* 1991). The rigid nature of the chelonian shell also aids endoscopic examination (Fig. 8.59).

To date endoscopy, in reptiles has not enjoyed widespread popularity, although there are numerous reports to indicate its use since the 1960s. The majority of previous papers describe the use of the endoscope to examine or retrieve foreign objects from the gastrointestinal tract (Coppoolse & Zwart 1985; Lumeij & Happe 1985; Ackermann & Carpenter 1995; Schildger 1997). There are some descriptions of laparoscopy and bronchoscopy, and more general descriptions of practical reptile endoscopy, particularly in chelonians (Wood *et al.* 1983; Schildger & Wicker 1992; Göbel & Jurina 1994; Kraut 1995; Jenkins 1996; Divers 1998a). The use of endoscopy for sex determination in monomorphic species and for internal examination of the urogenital system is well documented (Schildger 1994).

EQUIPMENT

Flexible endoscopes

Flexible endoscopes are most useful for gastrointestinal endoscopy (Fig. 8.60). The flexible scopes with uni- or bi-directional tips enable the clinician to examine the entire stomach, negotiate the pylorus, and enter the duodenum and small intestine. Likewise, a cloacal approach can permit the examination of the colon. Occasionally, when dealing with larger chelonians, a flexible scope may also be useful for examining the primary bronchi and lungs.

The main disadvantage of the flexible fibre-optic endoscope is the poorer image quality compared to rigid telescopes of similar diameter. A 100 cm, 2.5 mm flexible bronchofibrescope with 1.2 mm instrument channel is the authors' preferred instrument. Semi-rigid 1.3 mm scopes are also extremely valuable for examining the trachea of small specimens, weighing as little as 150 g.

Fig. 8.59 The author's (SH-D) endoscopy suite.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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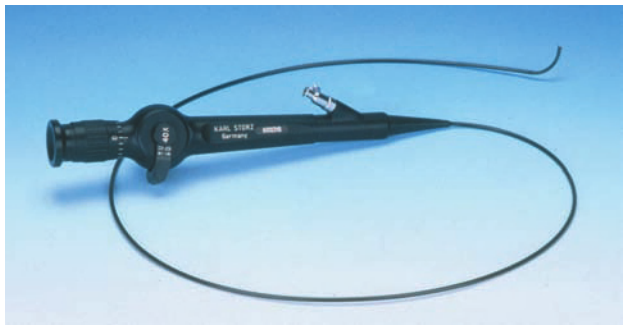

Fig. 8.60 Flexible endoscopes utilise flexible fibre optics for the transmission of light. This flexibility is ideal for gastrointestinal examinations.
(© Karl Storz GmbH & Co.)

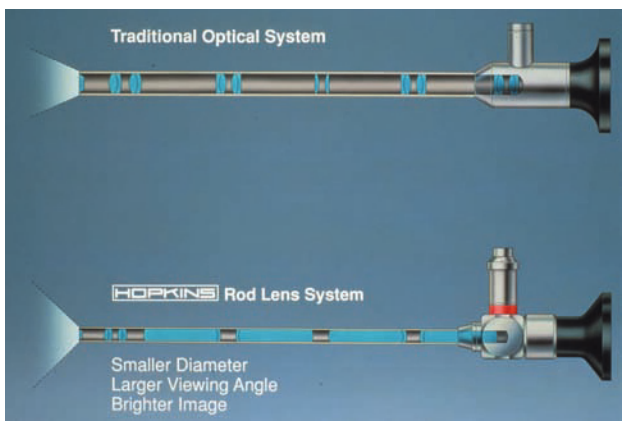

Fig. 8.61 Comparison between the conventional optical lens endoscope and Hopkins rod lens telescope. The comparatively longer rod lenses offer a significant improvement in optics.
(© Karl Storz GmbH & Co.)

Rigid endoscopes

Chelonians are ideal candidates for rigid endoscopy because of their compact body size and their rigid shell. A rigid scope can be used to examine the upper respiratory and gastrointestinal tracts in animals weighing 150–3000 g via an oral approach. The vent approach permits the examination of the cloaca (coprodeum, urodeum and proctodeum), distal colon and bladder.

The majority of older endoscopes incorporate a classic convex lens system, in which several small glass lenses are separated by large air spaces. A newer development, the rod lens telescope, invented by Professor Hopkins, utilises longer rods of glass and much smaller air spaces (Fig. 8.61). This advancement greatly improves the optical quality of the telescope and has the advantages of greater light transmission, better image resolution, wider field of view and image magnification.

Many endoscopic systems are available. The Storz 2.7 mm Hopkins telescope possesses a 30° oblique view that provides a greater field of vision. Many accessories are available to use with this scope, such as an operating 14.5 French (F) sheath. The operating sheath possesses a 5F instrument channel and two side ports for insufflation or irrigation (Figs 8.62, 8.63). A variety of endoscopic instruments are available, including biopsy forceps, scissors, aspiration/infection needle and retrieval forceps (Fig. 8.64).

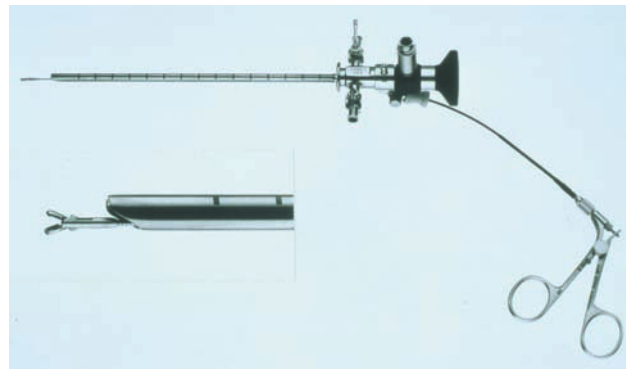

Fig. 8.62 The 2.7 mm telescope is protected within a 14.5F sheath. The operating channel permits the use of various instruments and so the entire endoscopy procedure can be accomplished via a single surgical approach.
(© Karl Storz GmbH & Co.)

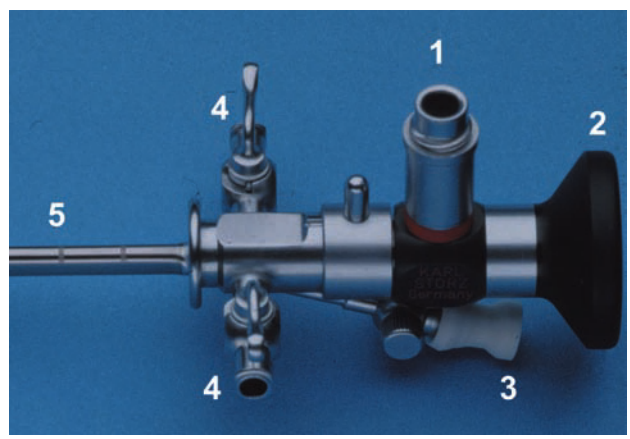

Fig. 8.63 Proximal close-up of the 2.7 mm telescope within the 14.5F sheath.
1—light-source attachment
2—eye-piece/video attachment
3—instrument channel
4—two insufflation ports
5—sheath body housing the telescope.
(© Karl Storz GmbH & Co.)

The flexible scissors and biopsy forceps enable the endoscopist to harvest tissue samples for histopathology and microbiology. The small sample size permits the taking of several and sequential biopsies to monitor progress in very small patients (<200 g). The grasping forceps are useful for tissue manipulation, debridement and foreign-body retrieval. The fine aspiration/injection needle can be used for remote aspiration, irrigation and drug administration (Fig. 8.64).

Light sources, cameras and recording equipment

There are two major types of light source available, the cheaper tungsten-halogen and the more expensive rare-earth xenon. Halogen is sufficient for rigid endoscopy using the eyepiece, but the xenon source is preferable for endovideo recording. More powerful light sources are preferable when dealing with the poorer optics of fine flexible endoscopes or larger animals.

The light source is connected to the endoscope via a flexible, fibre-optic cable. The efficiency of light transmission decreases as

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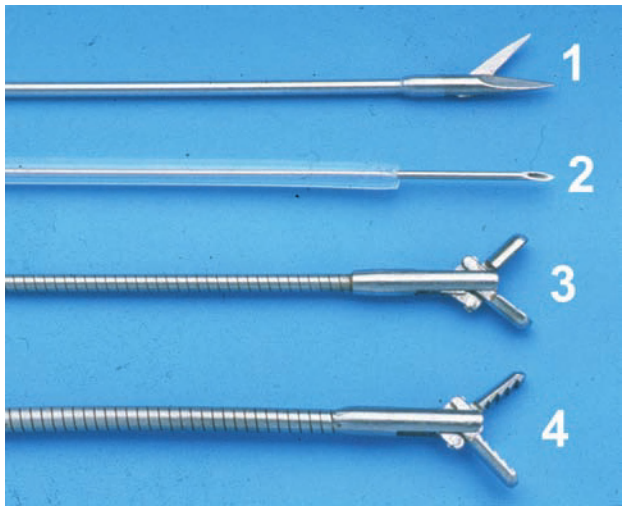

Fig. 8.64 Close-up of endoscopy instruments commonly employed with the 2.7 mm telescope.
(1) single-action scissors
(2) Teflon-coated needle
(3) biopsy forceps
(4) grasping forceps
(© Karl Storz GmbH & Co.)

Fig. 8.65 Preparation for reptile endoscopy. Note that the endoscopy equipment, including camera, light source, insufflator, monitor, digital video and still recording devices, are located on a mobile tower. This makes the entire unit portable and facilitates positioning to the surgeons preferences.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

cable length increases. The authors currently prefer the use of a xenon light source with a dedicated endoscopy camera and video recording capability for client education (Fig. 8.65).

EQUIPMENT AND PATIENT PREPARATION

When using the scope via the oral or cloacal entry routes, sterilisation is seldom necessary unless microbiological samples are to be collected. Nevertheless, the equipment should be clean. Asepsis is required especially when introducing the scope into the upper respiratory system (Fig. 8.66).

Coelioscopy necessitates proper sterilisation. Autoclaving is seldom recommended, although autoclavable telescopes are

Fig. 8.66 Endoscopy and minimally invasive techniques are surgical procedures that necessitate appropriate aseptic techniques.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

now available. The two practical options are gas sterilisation using ethylene oxide or cold sterilisation using a 2% glutaraldehyde solution followed by rinsing in sterile saline. The patient and surgeon must also be aseptically prepared and the use of sterile gloves, hats, masks and drapes should be considered mandatory.

ENDOSCOPY TECHNIQUES

Restraint, positioning and entry site preparation

General anaesthesia is recommended for all endoscopy procedures, although it is possible to scope the cloaca or oesophagus briefly in a conscious, physically-restrained tortoise.

For most procedures, induction using intravenous or intraosseous propofol followed by intubation and maintenance on isoflurane and oxygen (delivered by an electrical ventilator) has proven a safe and effective anaesthetic regime, particularly when dealing with critically-ill animals. Monitoring is also extremely important as insufflation can have significant effects upon the respiratory and circulatory systems. The use of Doppler ultrasound and pulse oximetry is preferred over a simple ECG, which will be affected by limb movement induced by breathing and insufflation.

With the animal adequately anaesthetised, the reptile can be appropriately positioned, depending upon the route of entry. For a buccal approach to the gastrointestinal and respiratory tracts, ventral recumbency with the head and neck extended is required. A similar technique can be used for examination of the cloaca, bladder and lower gastrointestinal tract with the patient in dorsal or sternal recumbency.

Precise positioning for coelioscopy depends upon the type of chelonian, the structure(s) of particular interest and the preference of the endoscopist. In most cases, a left prefemoral approach is preferred. The subject is positioned in right lateral recumbency with the left hind limb retracted and taped caudally (Fig. 8.68). The prefemoral fossa is then aseptically prepared using povidoneiodine and surgical spirit before a clear adhesive drape is applied over the entire area (Fig. 8.69)

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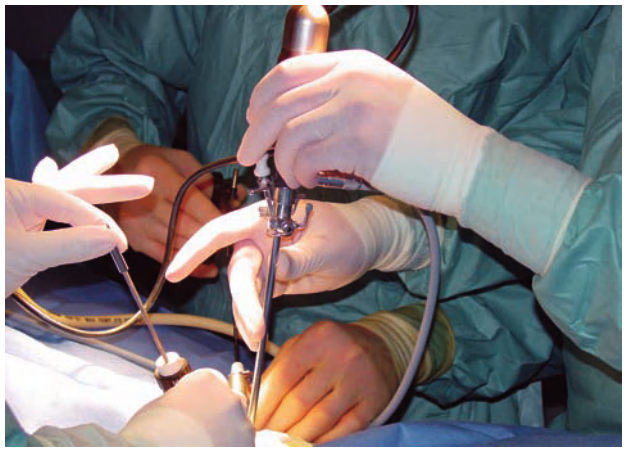

Fig. 8.67a Minimally-invasive endosurgical techniques are currently being developed at the University of Georgia. Given the restricted access afforded by the chelonian shell, these multiple entry techniques hold great promise for these species. In this three-port entry you can see the use of a telescope with camera attached, a palpation probe, and in the background, a pair of forceps also being used.

In chelonians it is often unnecessary to continually insufflate using carbon dioxide—in many cases a single injection of air will suffice as long as the endoscopist maintains a good seal around the instruments. (Courtesy of Stephen Hernandez-Divers, University of Georgia)

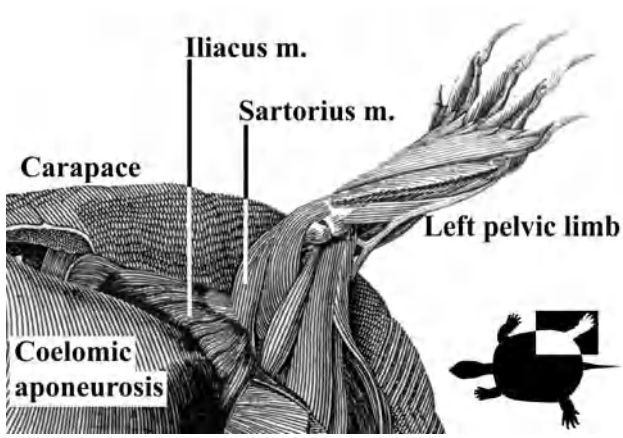

Fig. 8.67b The entry point for coelioscopy in chelonians is through the coelomic aponeurosis in the prefemoral region. Careful blunt dissection in the central prefemoral region, just cranial to the sartorius and iliacus muscles, will facilitate entry into the coelom (adapted from Bojanus, 1819). (Courtesy of Stephen Hernandez-Divers, University of Georgia)

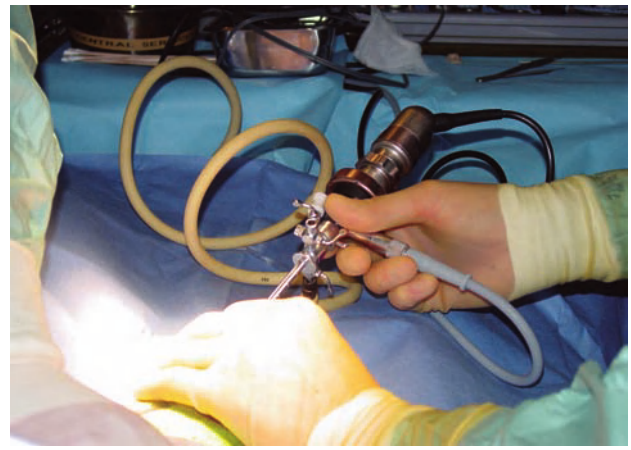

Fig. 8.67c The general endoscopy technique relies upon directing and supporting the end of the telescope with one hand while supporting the weight of the scope and camera with the other. Depending upon the conformation of the shell, right-handed surgeons generally find it easier to endoscope the left prefemoral fossa, and vice versa. The grey cable is the fiber-optic light source and the yellow is the insufflation tubing. (Courtesy of Stephen Hernandez-Divers, University of Georgia)

Fig. 8.67d The monitor should always be positioned directly ahead of the primary endoscopist to avoid poor ergonomics and fatigue. Here the primary endoscopist has an excellent view but the assistant has to turn to view the image.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

Endoscopic approaches to various organs

Table 8.5 summarises the approaches to the various sites.

Coelioscopy

The advantage of coelioscopy over exploratory coeliotomy is that as a minimally invasive procedure only a single skin suture is required to close the surgical wound (Fig. 8.70). Taking aseptic precautions, a small skin incision is made in the centre of the prefemoral fossa. A pair of small haemostats can then be introduced and directed cranially, avoiding the dorsally situated lungs, until the tough coelomic membrane is breached (Fig. 8.72). Just as when introducing haemostats into the caudal thoracic air sac of birds, there is often a detectable 'pop' as the haemostats break through the membrane.

Continuous insufflation is rarely required in chelonians; however, a single injection of air (typically 30–40 ml/kg) via one of the sheath ports is often required to provide good visualisation of the coelomic cavity and viscera where continuous insufflation is required a dedicated CO_2 endoflator set to 2–4 mmHg should be considered. In all cases, careful thought must be given to the effects of insufflation on lung ventilation and respiration. The use of pressure-sensitive ventilation and pulse oximetry can be extremely valuable to monitor circulatory and respiratory changes. Various gases can be used for insufflation, namely carbon dioxide, nitrous oxide and air. A variety of specialised gas supplies, filtration units and insufflation devices are available,

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Table 8.5 Endoscopic approaches.

Site	Approach	Comments
Upper digestive tract, oesophagus, stomach, pharynx and trachea	Reached by oral approach.	Limited immobilisation and instrument sterilisation required.
Upper respiratory tract	Caudocranial approach via an oesophagostomy incision. Alternatively the use of fine 1 mm or 1.3 mm scopes via the nares of most adults.	Allows biopsy of choana in cases of upper respiratory tract disease.
Liver	Approach through the prefemoral fossa.	Often the first organ encountered. Blunt edge extends caudally towards prefemoral fossa along the inside of the carapace. Normally dark brown, may be yellow in lipidosis in which case melanin pigment granules also become apparent. Common site for biopsy.
Septum horizontale	Approach through the prefemoral fossa.	Underside of membrane forms roof of coelom. Transparent to opaque, pink to dark-coloured.
Lungs	The lungs can be examined by a caudal approach through a coeliotomy incision at the junction of the septum horizontale membrane, or through a temporary carapacial osteotomy.	Allows sampling of localised pulmonary lesions, biopsy and therapy.
Small intestine	Approach through the prefemoral fossa.	Pale pink with superficial blood vessels. Inspect for intussusception or obstruction.
Large intestine	Approach through the prefemoral fossa.	Larger diameter, thin-walled and with darker content.
Stomach	Approach through the prefemoral fossa.	Large, smooth, pink structure in left cranial abdomen.
Pancreas	Approach through the prefemoral fossa.	Best seen from the right side. Pale yellowish strip alongside duodenum.
Spleen	Approach through the prefemoral fossa.	Discrete round organ in central cranial coelom. Often beneath small intestine. Common site for biopsy.
Cloaca	The lower digestive and urogenital tracts can be examined via the cloaca.	May require chemical restraint in larger animals. A constant fluid drip can be used to distend the cloaca for better visualisation (as done in birds).
Bladder	Approach through the prefemoral fossa or via the cloaca.	Thin-walled and fluid-filled. Urate crystals may be visible in content. Cystocentesis is relatively easy.
Kidney	The kidneys can be approached retrocoelomically through the prefemoral fossa.	May be difficult to visualise at extreme caudodorsal limit of field of view unless using an oblique scope of 30° or more. Dark brown to black. Ureter, blood vessels and testes must be avoided at biopsy.
Ovary	Approach through the prefemoral fossa.	Beneath kidneys in immature animals. Striking orange follicles of variable size occupy space in front of bladder. Vast numbers present in pathological follicular stasis. Oophoritis may also be observed.
Testes	Approach through the prefemoral fossa.	Globular, yellow-orange organs adherent to kidney.
Heart	Approach cranially via a cervicobrachial approach, or caudally from a prefemoral approach.	Identified by obvious beating. Beneath liver cranially.

but the authors have never experienced any problems with the use of room air.

Insertion of the scope will often result in an initial blurred image due to the presence of tissue fluid on the terminal lens. Simply touching or gently wiping the end of the scope against a serosal surface will clean the lens, greatly improve visibility and permit orientation within the coelom for subsequent examination of the viscera (Fig. 8.73). The first organ likely to be encountered is the liver (Fig. 8.74).This is a large organ, typically dark red to brown in colour, and once it is located the endoscopist can locate most if not all of the organs of interest (Figs 8.73–8.91) (Table 8.6).

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Fig. 8.68 The left hind limb is taped caudally exposing the prefemoral fossa, which must be thoroughly and aseptically prepared and draped. The tortoise is supported in lateral recumbency using sandbags.
(© Stephen Hernandez-Divers, 1999)

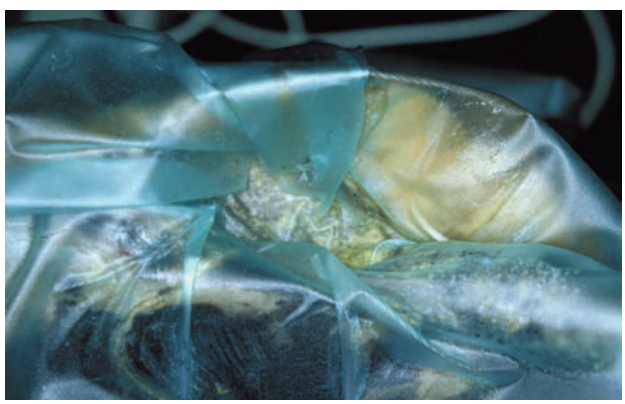

Fig. 8.69 The use of clear, adhesive drapes greatly improves the visualisation of the area, which facilitates correct surgical entry through the ventral aspect of the fossa.
(© Stephen Hernandez-Divers, 1999)

Fig. 8.70 A single skin suture is all that is required to close the endoscopy entry wound.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

Gastroscopy

Rigid endoscopes can be used to examine the gastrointestinal tract from the buccal cavity in chelonians less than 2 kg, however flexible scopes are usually required for larger specimens (Fig. 8.92).

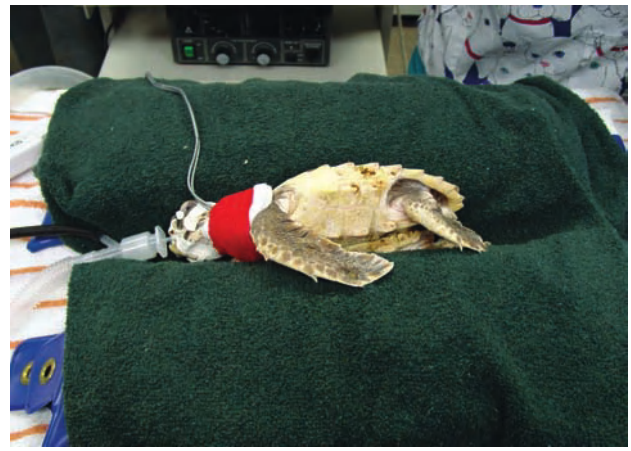

Fig. 8.71 Positioning a yearling loggerhead sea turtle (*Caretta caretta*) in preparation for prefemoral coelioscopy. The turtle is intubated and maintained using a ventilator, and monitored using carotid (Doppler flow) pulse strength and rate.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

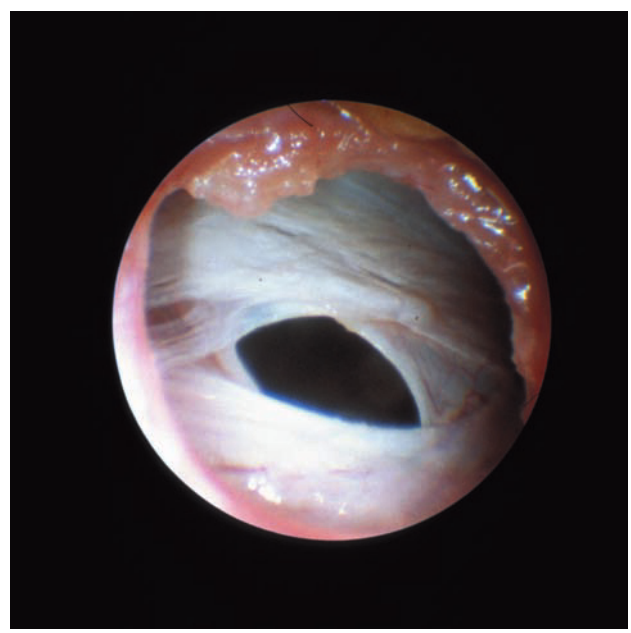

Fig. 8.72 Endoscopic view of the fibrous coelomic membrane that must be breached using small haemostats in order to gain entry into the coelomic cavity (*Geochelone sulcata*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

Air insufflation is useful to dilate the alimentary tract and aid foreign body retrieval and identification of gross lesions. When examination of the delicate mucosa is necessary, the use of sterile saline is preferred over air because it permits a better appreciation of the mucosal architecture.

A litre bag of sterile saline (warmed to 28°C) is suspended above the examination table and an intravenous giving set is used to connect this bag to one of the ports of the sheath. A second giving set is connected from the other sheath port to a collecting bowl under the examination table. By controlling both inflow

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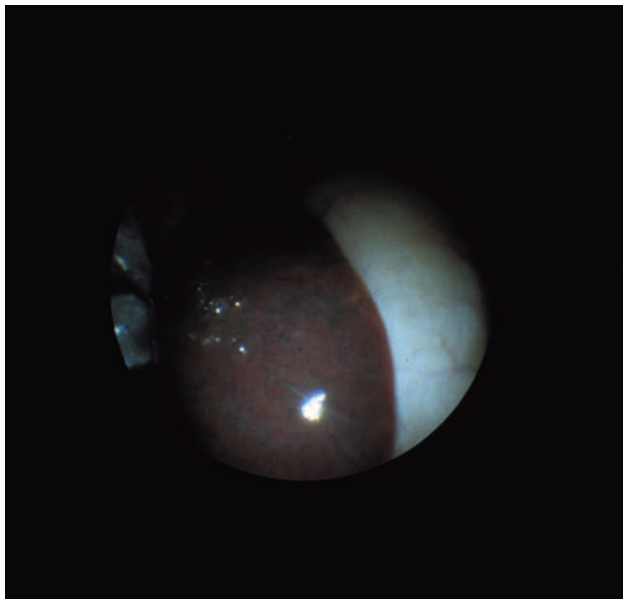

Fig. 8.73 Initial endoscopic view is often blurred by tissue fluid on the terminal lens. This can be removed by gently touching the tip of the scope against a serosal surface. The first organ likely to be seen is the large liver and stomach. In this photograph, a normal, dark red-brown liver can be seen bordered by the paler stomach (*Testudo graeca*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

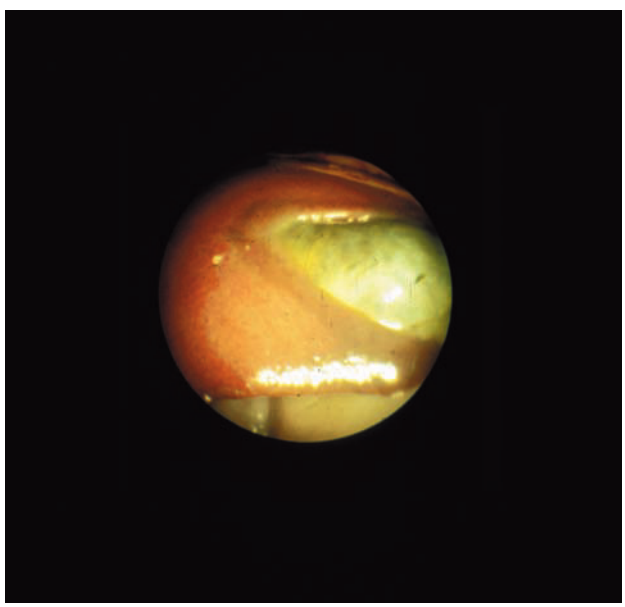

Fig. 8.74 Coelioscopic view of a normal liver and gallbladder (*Testudo graeca*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

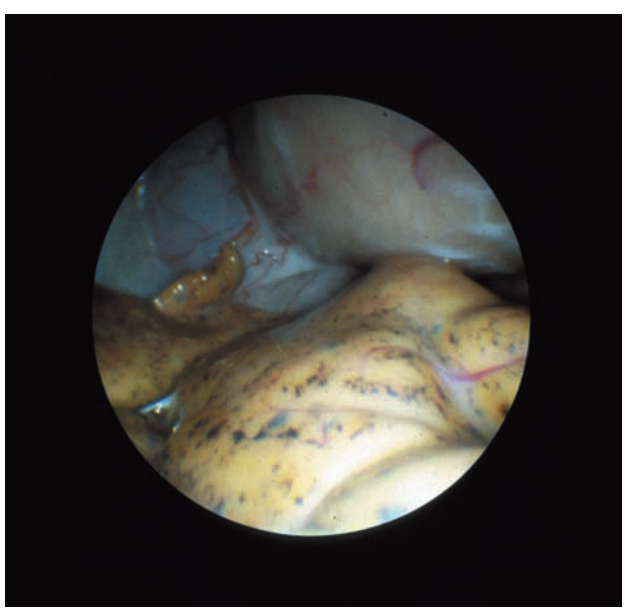

Fig. 8.75 Coelioscopic view of an enlarged pale liver. Note the normal multifocal areas of melanin pigmentation (*Testudo graeca*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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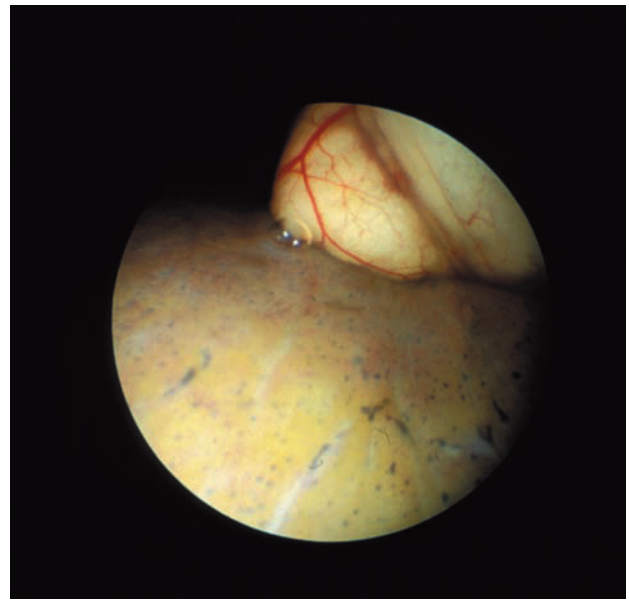

Fig. 8.76 Coelioscopic close-up view of a swollen pale liver lobe. In this case, the female was reproductively active and producing follicles (top right) which resulted in a great increase in cholesterol and triglyceride metabolism and a physiological hepatic lipidosis (*Testudo hermanni*). (Courtesy of Stephen Hernandez-Divers, University of Georgia)

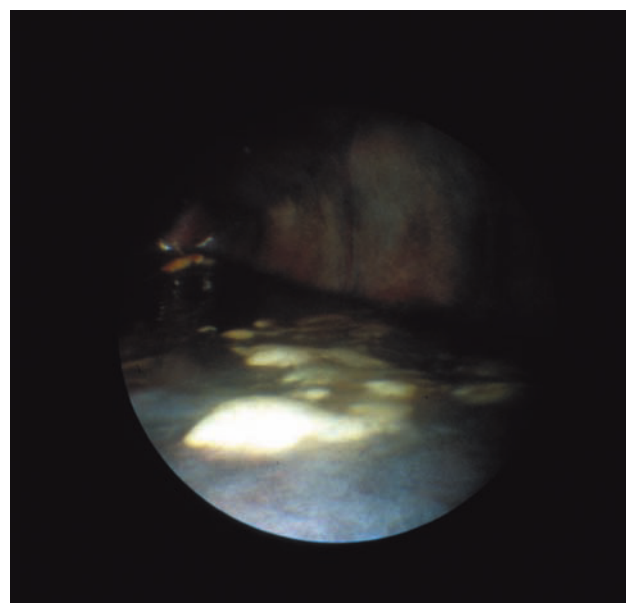

Fig. 8.77 Coelioscopic view of the surface of a dark liver with obvious pale plaques. These were identified on biopsy as fungal granulomata (*Testudo graeca*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

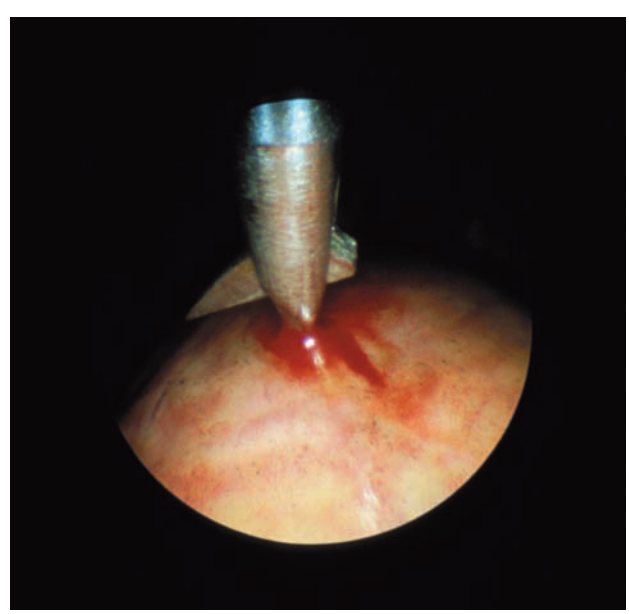

Fig. 8.78 The collection of endoscopic visceral organ biopsies (in this case liver) is greatly facilitated by first incising the serosal membranes using dedicated endoscopy scissors (*Trachemys scripta elegans*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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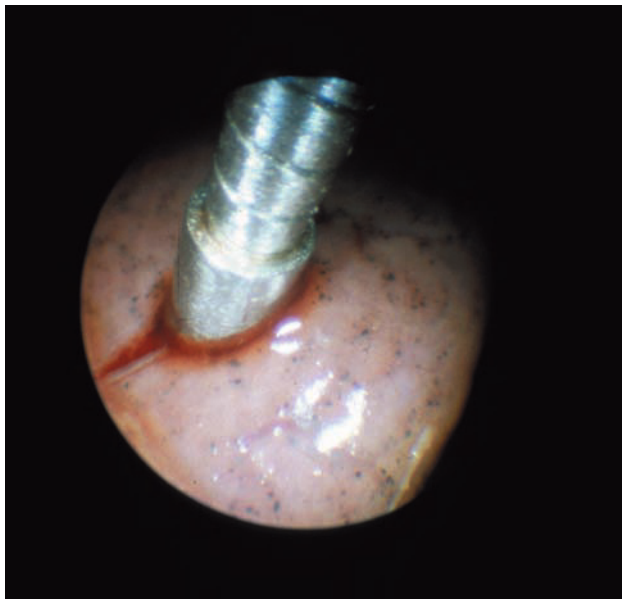

Fig. 8.79 Following membrane incision, the biopsy forceps can be introduced under the capsule to enable the harvesting of a clean tissue sample (liver; *Testudo kleinmanni*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

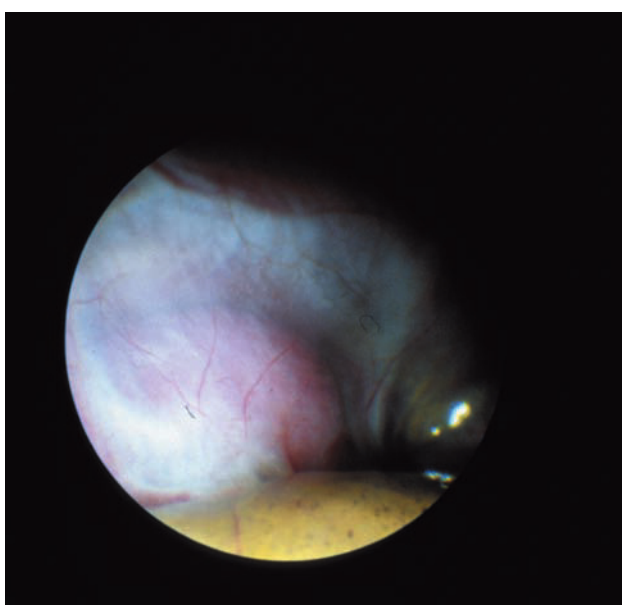

Fig. 8.80 Coelioscopic view of the heart and pericardium (*Geochelone pardalis*). Occasionally, urate deposits or effusions that can be endosurgically removed may be seen within the pericardium.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

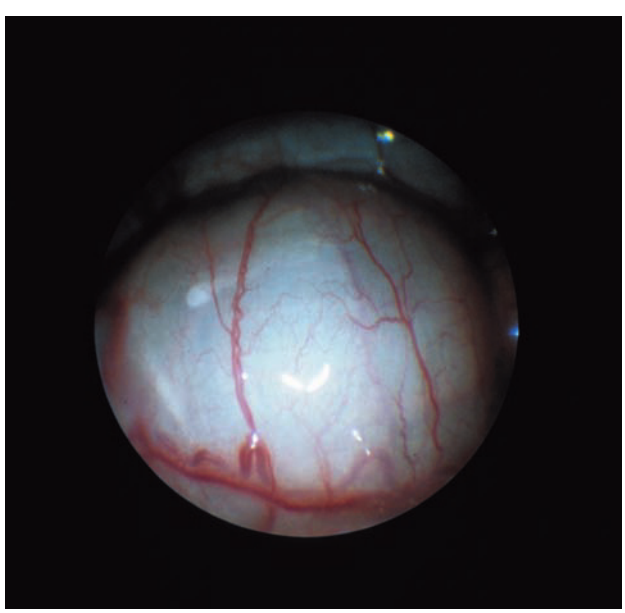

Fig. 8.81 Coelioscopic view of a distended stomach (*Testudo graeca*). Note the moderate engorgement of the gastric vasculature.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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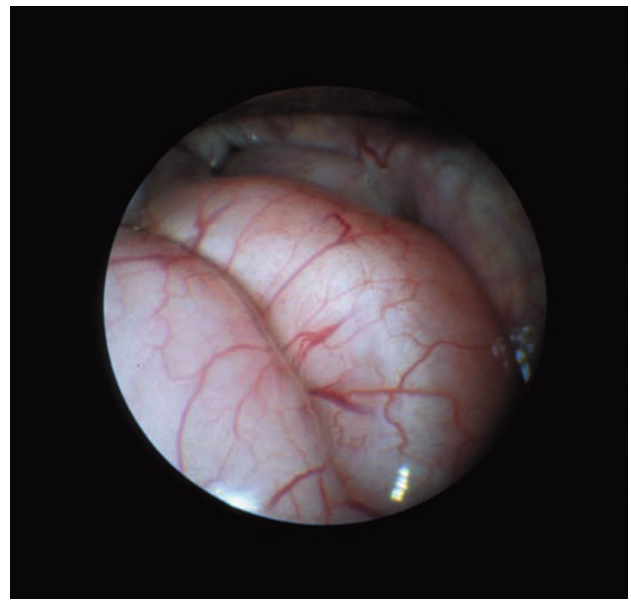

Fig. 8.82 Coelioscopic view of distended loops of small intestine due to enteritis associated with feeding highly fermentable fruits (*Testudo graeca*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

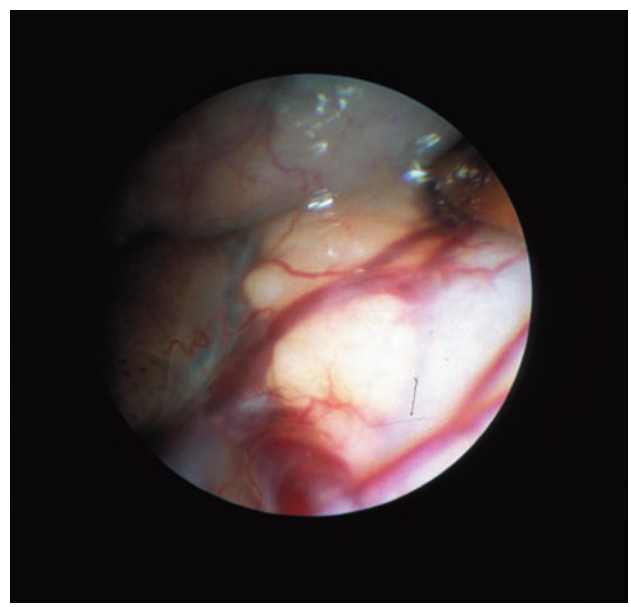

Fig. 8.83 Coelioscopic view of a normally pale pancreas (*Testudo graeca*). Suspected diabetes mellitus should be confirmed by pancreatic biopsy.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

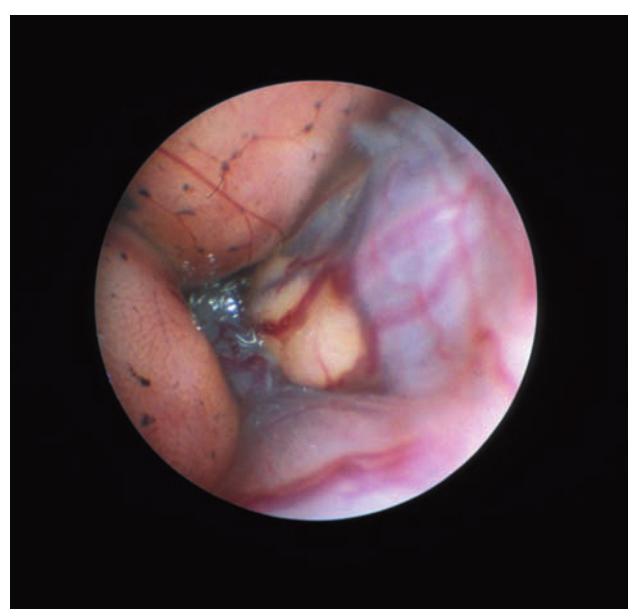

Fig. 8.84 Coelioscopic view of a pale spleen (centre) from an anaemic Hermann's tortoise (*Testudo hermanni*). Endoscopic biopsy revealed diffuse and severe fibrosis. Also note the pale adjacent liver.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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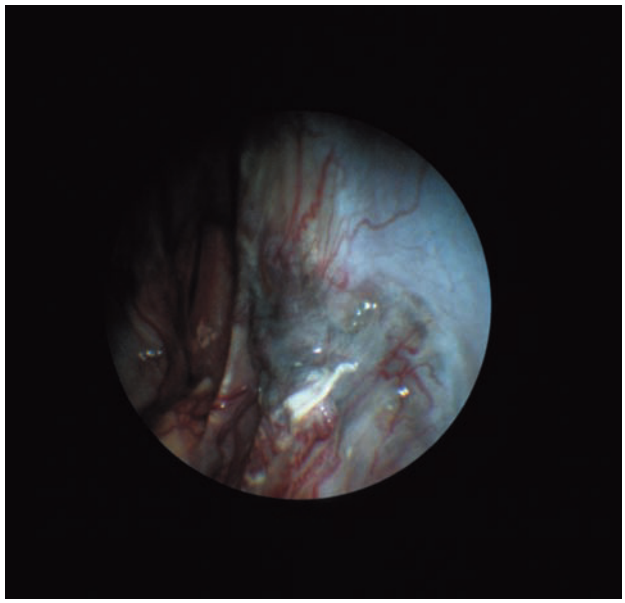

Fig. 8.85 Coelioscopic view of the pleuroperitoneum, the thin membrane that separates the lungs from the ventral coelomic viscera (*Testudo graeca*). Entry into the lungs via the pleuroperitoneum is generally contraindicated due to the dangers of creating a pneumocoelom.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

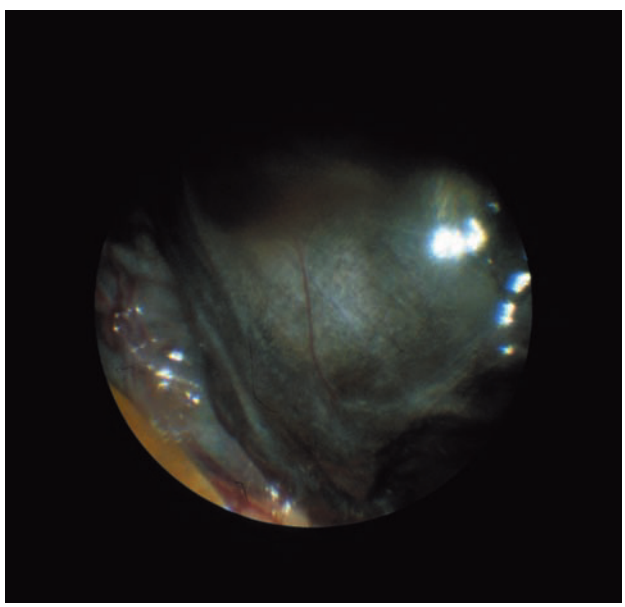

Fig. 8.86 Coelioscopic view of a normal kidney in a female tortoise (*Testudo graeca*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

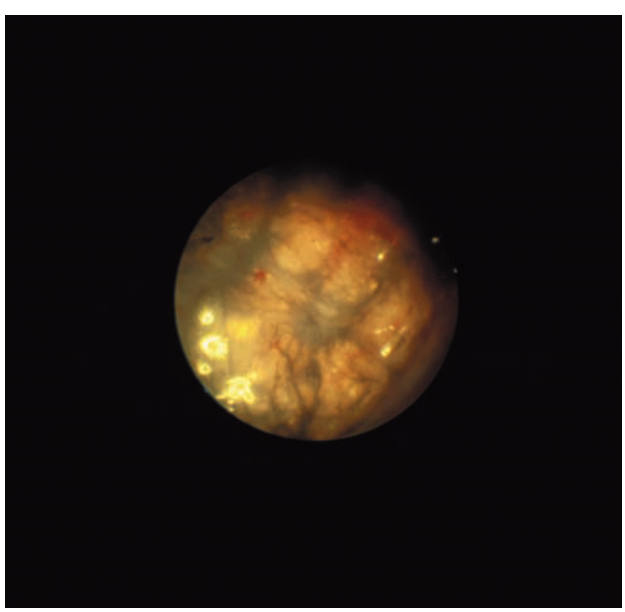

Fig. 8.87 Coelioscopic view of an abnormal kidney demonstrating obvious subcapsular urate deposits and petechiation (*Testudo graeca*). In such cases biopsy may well provide a definitive diagnosis and prognosis.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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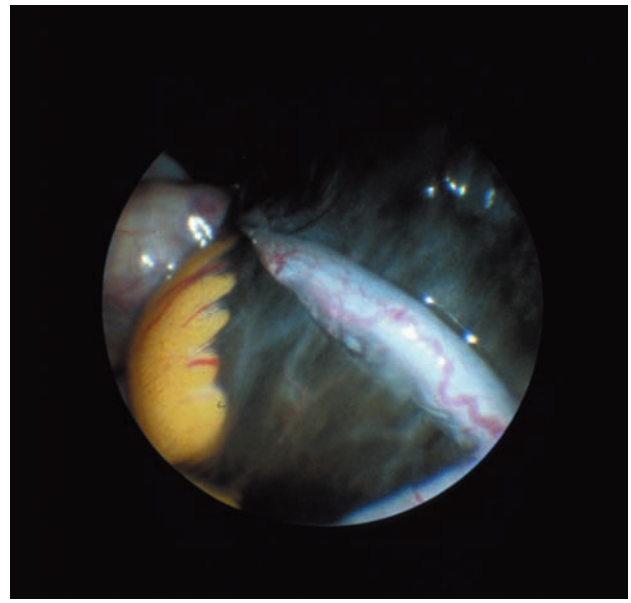

Fig. 8.88 Coelioscopic view of a normal kidney and underlying yellow testis. Note the ureter and vas deferens coursing across the surface of the kidney (*Testudo hermanni*).
(Courtesy of Stephen Hernandez-Divers, 1999)

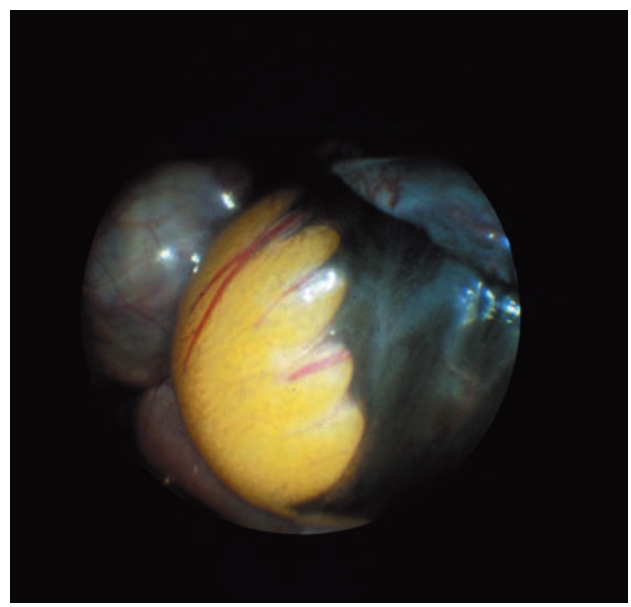

Fig. 8.89 Coelioscopic view of a normal testis (*Testudo hermanni*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

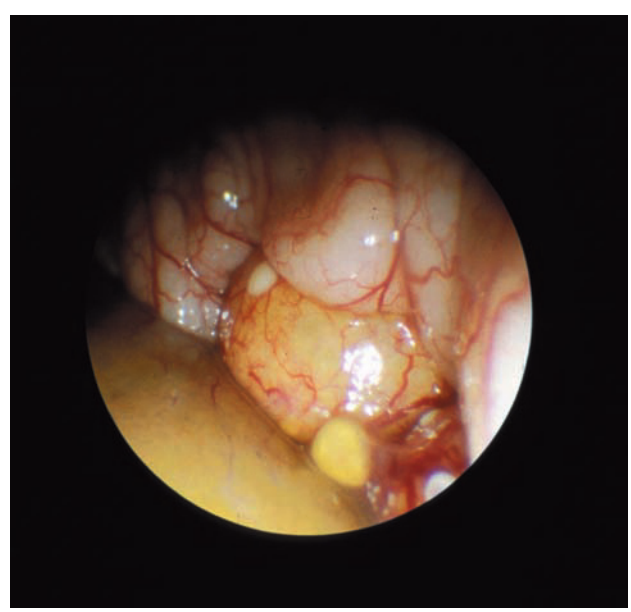

Fig. 8.90 Coelioscopic view of the left ovary, which is largely obscured by the closely-associated infundibulum (*Testudo graeca*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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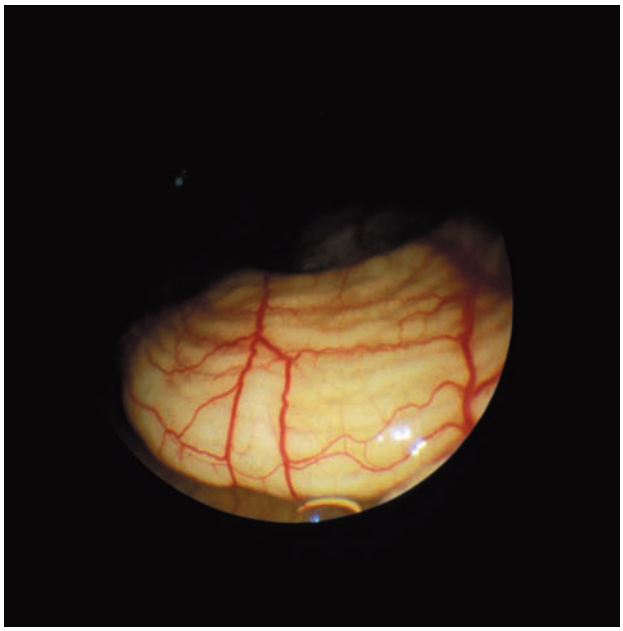

Fig. 8.91 Coelioscopic view of the left shell gland (*Geochelone gigantea*). Note the corrugated appearance of this structure and the lack of distension, due to the absence of eggs.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

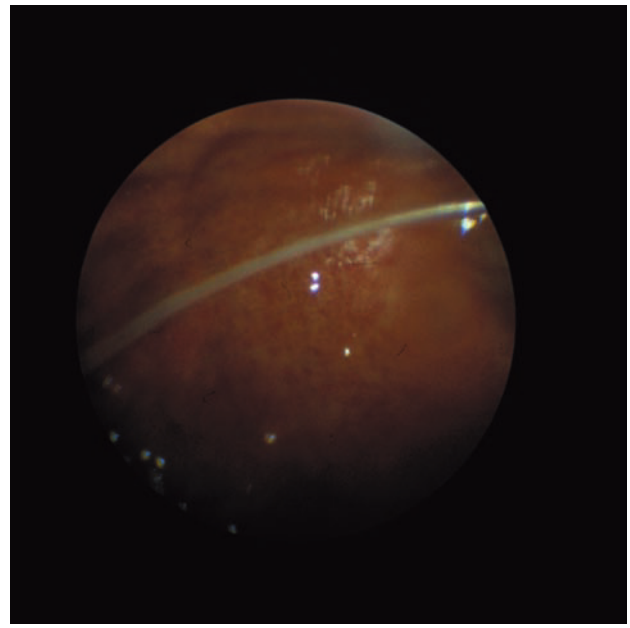

Fig. 8.92 Endoscopic view of the stomach via a buccal approach (*Geochelone pardalis*). In this case, the remains of a bitten-off and swallowed dosing catheter can be seen. This was successfully removed using grasping forceps negating the need for invasive surgery.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

Table 8.6 Endoscopic appearance of visceral organs.

Organ	Appearance	Position	Comments
Liver (Figs 8.73–8.79)	Normally dark red to brown but pale tan to yellow in cases of hepatic lipidosis (physiological and pathological). Surface usually smooth. Irregularities often represent fibrosis, granulomata, neoplasia etc.	Cranioventral coelom. May extend far caudally, making biopsy from the caudal edge difficult.	Large organ, easy to biopsy but care must be exercised to avoid the gall bladder.
Heart (Fig. 8.80)	Unmistakable muscular, red, beating structure.	Located in the cranioventral aspect of the coelom.	Avoid damage but aspiration of pericardial effusions using the Teflon-coated needle is relatively straightforward.
Stomach (Fig. 8.81)	Generally a pale, smooth structure with superficial blood vessels that become greatly engorged in cases of bloat or gastroenteritis.	Located within the left central area of the coelom but often displaced dorsal to the liver during lateral recumbency for endoscopy.	There is little interest in the serosal surface of the stomach except to check for perforations.
Small intestine (Fig. 8.82)	Tubular, pale structure, with superficial blood vessels that engorge in cases of ileus, enteritis, and bloat. Intussusception and blockage can be readily appreciated.	Caudal to the liver and stomach, and normally in a ventral position. During endoscopy tends to be displaced under the liver and stomach. Better visualisation from a right prefemoral approach.	
Pancreas (Fig. 8.83)	Diffuse, pale, cream to yellow structure. Erythema and petechiation associated with pancreatitis. Care is required to avoid iatrogenic trauma of this sensitive tissue.	Located along the duodenum. Generally best visualised from a right prefemoral approach.	Biopsy essential to confirm diabetes mellitus.

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Table 8.6 (*cont'd*)

Organ	Appearance	Position	Comments
Spleen (Fig. 8.84)	Pink to light brownish-red in colour. Gross changes seldom documented.	Bordered by the greater curvature of the stomach and duodenum but variable location in lateral recumbency. It is often necessary to displace small intestine to gain access to the spleen.	Excellent biopsy site for diagnostic of viral disease and lymphocytic disorders.
Large intestine and colon	Larger-volume tubular structures, similar to stomach but darker in colour. Visibly thin walled and lacking the obvious superficial vasculature.	Caudal position makes visualisation difficult.	Thin walled, avoid damage.
Kidney (Figs 8.86–8.89)	Generally dark red/brown to almost black in colour, irregular in appearance, with the ureter usually visible coursing across the surface. Generally overlying the testes of the male and may well envelope epididymal tissue.	Retro-coelomic, caudodorsal position, closely adherent to the carapace. Considered impossible to visualise by some but certainly accessible in most cases using an oblique scope. Only one organ may be seen from either lateral approach.	Great care is required not to damage the large superficial blood vessels during biopsy procedures. Cords of pale epididymal tissue are often visible through the renal parenchyma.
Bladder	Very variable in size but generally thin walled with tortuous superficial vasculature and ligamentous urachal attachment to central plastron.	Caudal to mid-coelom, often located in the most dependent aspect of the coelom during endoscopy.	Aspiration using the Teflon-coated needle is simple and provides a safer cystocentesis sample with greatly reduced risk of bladder laceration or haemorrhage.
Testes (Figs 8.88–8.89)	Spherical, pale, yellow to vivid orange in colour.	Partially obscured by the kidney. Only one can be seen from either lateral approach.	Biopsy of the testis is certainly possible and may be of use in cases of reduced fertility.
Ovary (Fig. 8.90)	Grape-like structures consisting of follicles varying in size and colour (yellow to orange). Follicular absorption often characterised by engorgement of follicular vasculature.	Suspended from dorsolateral ovarian ligaments, the ovaries usually lie in the mid-coelomic region, but lateral recumbency causes dorsal displacement. Only one can be seen from either lateral approach.	
Oviducts, shell glands (Fig. 8.91)	Flattened structures with a corrugated appearance that are cream to pale tan in colour when inactive. Distended with eggs during active egg production.	Generally located laterally in the mid- to caudal-coelomic region but lateral recumbency often displaces dorsally. Usually only one can be seen from either lateral approach.	Biopsy seldom warranted, but manipulation may be required to locate other structures. Proximal infundibulum is much more fragile than the robust distal shell gland.
Lung (Fig. 8.85)	Not directly visualised but the ventral surface of the septum horizontale membrane can be clearly seen in the dorsal aspect.	Dorsal coelom.	Entry into the lung is possible via a small incision made in the septum horizontale membrane, but this is not recommended due to the dangers of post-operative pneumocoelom unless the incision can be closed. It is recommended either to gain access at the insertion of the septum horizontale and the carapace, or to perform a carapacial osteotomy and insert the scope directly into the lung.

and outflow, the operator can infuse and aspirate saline, thereby providing a clean view of the alimentary tract and a detailed appreciation of the mucosa.

Similar techniques can be used to examine the cloaca and large intestine and, with practice, the bladder can also be entered via the urethral opening situated in the urodeum.

Pneumoscopy

The unsheathed scope can be used to examine the buccal cavity, glottis, trachea and proximal bronchus in many species (Figs 8.93–8.95). However, endoscopic entry into the lungs via the tracheobronchial approach is very difficult because there is

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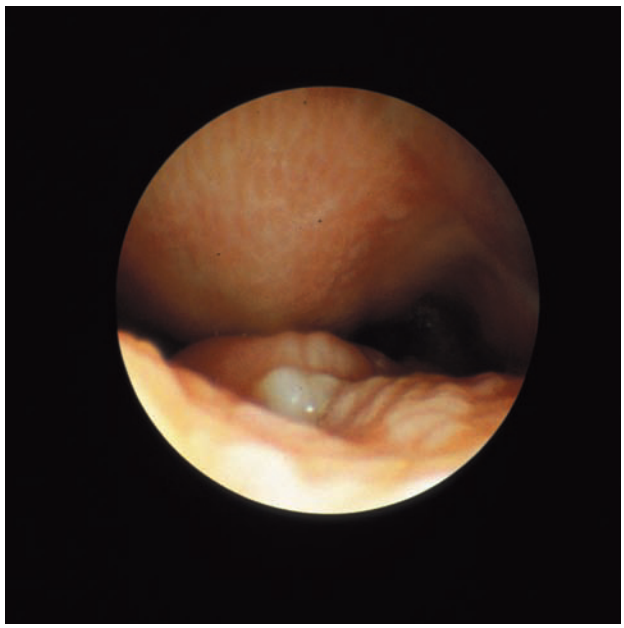

Fig. 8.93 Endoscopic buccal view of the glottis with the fleshy tongue situated ventrally (*Testudo graeca*).
(©Stephen J. Hernandez-Divers, 1999)

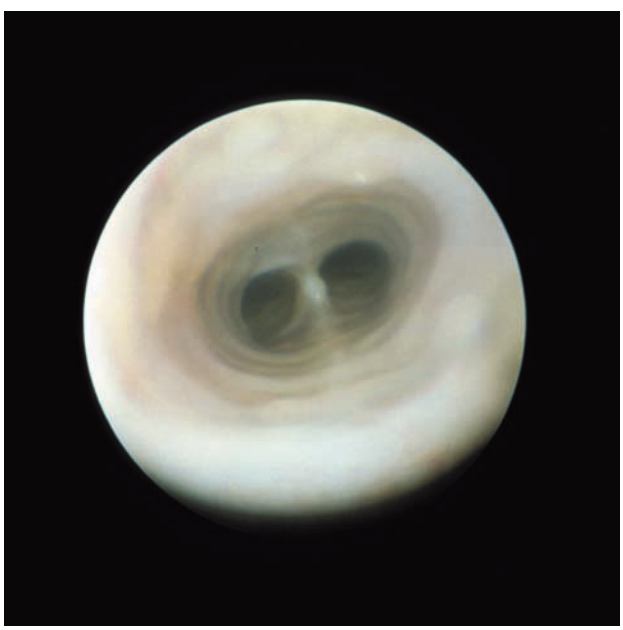

Fig. 8.94 Endoscopic view of the short trachea with the bifurcation in the distance (*Geochelone pardalis*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

Fig. 8.95 Endoscopic view of the tracheal bifurcation (*Testudo hermanni*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

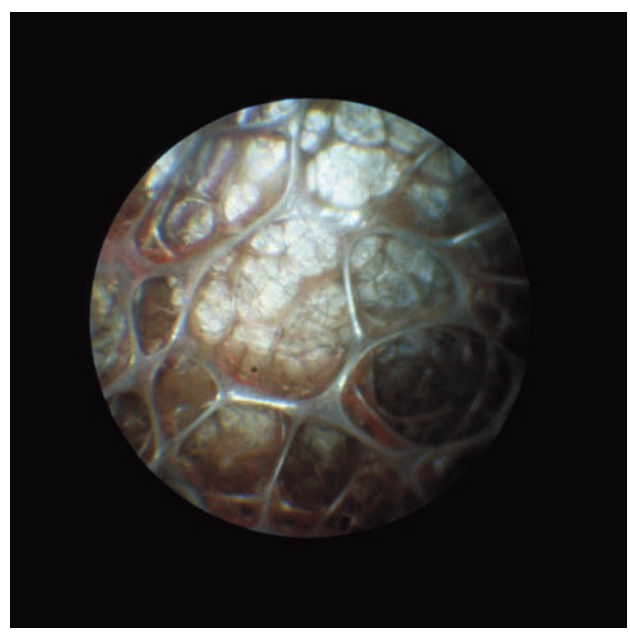

Fig. 8.96 Endoscopic view of the lung via a temporary hole drilled through the carapace. Note the coarse honeycomb structure of the lung. The collection of endoscopic aspirates and biopsies assists in making a definitive diagnosis (*Testudo graeca*).
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

an acute deviation in the primary bronchus before it enters the medial aspect of the lungs.

Entry into the ventral aspect of the lungs via a coelioscopic approach is certainly possible, but the problems of closing the septum horizontale and the danger of inducing a pneumocoelom are important considerations.

A safer and more practical means of examining the lung is via temporary osteotomy of the carapace (Fig. 8.96). Manoeuvrability is limited due to the restriction of the diameter of the hole, and so it is important to take radiographs to pinpoint the area of interest prior to endoscopic entry. The hole is simply covered with epoxy resin or an acrylic compound after the procedure (Divers 1998b).

Organ biopsy

One of the great benefits of endoscopy is that, when an abnormal structure or pathological lesion is observed, biopsies can be taken under direct visual control. Biopsies can be harvested from the kidneys, gonads, liver, spleen, pancreas, fat tissue, lung,

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gastrointestinal tract, coelomic membranes and, in general, any abnormal soft-tissue structure.

The visceral organs are covered by serosal membranes that can be difficult to break through using the delicate biopsy forceps. Furthermore, repeated bite attempts may have to be made before the membrane is breached and tissue obtained. The authors prefer to use flexible endoscopy scissors to incise the serosal membrane, which then permits the entry of the biopsy forceps and the collection of a clean tissue biopsy (Figs 8.78–8.79).

It is vital that, as clinicians, we correlate histopathological and microbiological biopsy results with clinicopathological data when dealing with reptile diseases. The use of biopsy forceps may be contraindicated when dealing with cystic structures due to the risks of post-sampling leakage. In such circumstances the Teflon-coated endoscopy needle can be used to aspirate from these deep structures with minimal risk. Likewise, remote injections are also possible.

SUMMARY

The benefits of endoscopy include visualisation, inspection and sampling procedures, gender determination of juvenile or monomorphic species, therapies including foreign body removal, endoscopic surgery and assessing response to treatment by serial examinations. Endoscopy permits entry and examination of gas and fluid environments (which sets it apart from ultrasonography) and clearly differentiates between closely-associated tissues (which sets it apart from radiography). Anatomical knowledge and practical experience are essential to become competent with the techniques that will help achieve ante-mortem diagnoses. To this end, the authors recommend practice on carcasses and attendance at practical endoscopy demonstrations and wet-labs.

MAGNETIC RESONANCE IMAGING (MRI)

(Ian Calvert)

Since its first clinical use in the 1980s, MRI has rapidly progressed in human medicine, from an esoteric research instrument to a powerful clinical tool that is displacing radiography, computer tomography, myelography, arthrography and even angiography. With its widespread use, veterinary access to MRI scanners is likely to increase in the near future. Although the current costs of MRI imaging are high compared to other imaging modalities, this is more than offset by the amount of diagnostic information that can be gathered in a relatively short period of time and with very little risk to the patient. As a method of imaging the enigmatic interior of the chelonian 'box' MRI is unrivalled. However its true potential will only be realised when sufficient clinicians have used the technique on enough animals to permit an accurate interpretation of the resultant images.

Key to the figures that accompany this section:
aorta **A,** left aorta **LA**, right aorta **RA,** right aortic arch **RAA**; Acromial process of scapula **Ap**; right pulmonary artery **ASA**; bladder **Bl**; bladder stone **BS**; brain **Br**; bronchus **B**; caecum **Ca**; cloaca **Cl**; caudal vena cava **CVC**; colon **C,** transverse colon **Ct,** distal colon Cd; duodenum **D,** descending duodenum **dD**; debris in bladder **db**; femoral cavity **F**; femoral head **Fh**; gall bladder **Gb**; glenoid cavity **Gc**; heart **H;** right auricle **RAr**; left auricle **LAr**; hind limb **HL**; ileum **I**; iliac artery **Iat**; innominate artery/base of carotid arch **IA**; iliofemoralis muscle **If**; ischium **Ic**; kidney **K**; right kidney **RK**; left kidney **LK**; liver **L**; left bronchus **LB**; lung **LG;** lesion **Ls;** neck **N;** metal artefact **Mta**; movement artefact **ma**; oesophagus **O**; ovulatory follicle **Of**; pericardium **Pc**; pubis **Pb**; rectum **R**; renal artery **Rat**; right aortic arch **RAA;** stomach **S**; spinal chord **SC**; scapula **Scp**; shell gland **SG;** suspensory ligament **SL**; ureter **Ur**; uric acid crystals **Urc**; ventricle **V**.

MRI PHYSICS

Although multi-cellular animals are opaque when viewed with low frequency radiation in the ultraviolet and visible light part of the spectrum, biological tissue is transparent to high-frequency, high-energy, electromagnetic radiation such as gamma and X-rays and it is this transparency which is utilised in conventional radiographic imaging techniques. MRI cleverly exploits the fact that animal tissues are similarly transparent to long-wave radio frequency radiation at the opposite end of the electromagnetic spectrum. The physics of MRI imaging are complex, but it is useful to have some appreciation of the terminology in order to communicate with the radiographer and understand the information printed on the resultant MRI radiographs (for a more detailed discussion the reader should refer to reviews such as those by Lufkin 1990 and Grodd 1998).

Magnetic resonance imaging makes use of the fact that atomic nuclei with unpaired protons or neutrons (unbalanced nuclei) can be made to 'wobble' when placed in a magnetic field. When these nuclei 'flip' from one energy state to another they emit a pulse of weak radio frequency (Rf) energy. This can be detected and used to construct an image of the tissues of a patient placed in the magnetic field. Although quite a number of elements have unbalanced nuclei, medical MRI makes use of hydrogen atoms. Hydrogen is particularly suitable for imaging because up to two thirds of all the atoms in biological systems are composed of this element.

To produce an MR image, the patient is placed inside the bore of a large electromagnet (Fig. 8.97). This is used to generate a large magnetic field that causes protons to align with the magnetic field like compass needles. However the protons do not line up precisely with the magnetic field, rather they spin

Fig. 8.97 Aldabran tortoise (*Dipsochelys elephantina*) being loaded into a Phillips Gyroscan MRI scanner.
(Courtesy of Ian Calvert)

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or 'precess' around the magnetic axis at a slight angle to the field, rather like the motion of a spinning gyroscope. The frequency of the spin is known as the resonant or Lamour frequency and is determined by the strength and frequency of the applied magnetic field, hence the term magnetic resonance imaging (MRI). In practice, it is primarily only the protons in water and fat that are mobile enough to be moved by the magnetic field changes.

An MRI scanner also contains radio frequency (Rf) transmitting and receiving coils. The waveform detected is complex, and computer-based analysis is used to translate the phase and amplitude of signals from different parts of the magnetic field into points on a greyscale. This digital data can then be displayed as a visual image.

By altering the timing and strength of the radio frequency pulses, it is possible to detect differences in the characteristics of protons within different parts of the tissue. The most widely used sequence is known as Spin-Echo.

Substances such as fat, proteinaceous fluids and lipid-rich molecules produce a bright (white) image in T1-weighted scans (high signal). Paramagnetic substances such as methaemoglobin and melanin also shorten T1 so that areas of haemorrhage and melanomas also show up as a bright intensity on T1-weighted images.

Any kind of cellular change that alters the properties of cellular membranes and increases bulk (unbound) water changes T1 and T2 images. Tumours, inflammation, oedema, pure fluids and CSF produce black, T1-weighted image areas. Conversely these pathological changes result in bright (white) areas on T2-weighted images. However T1 and T2 weighted images are not simply the inverse of each other. By using both types of images it is possible to detect subtle changes within organs (compare the faeces in the colon in Figs 8.98–8.99).

Modern computing techniques have allowed numerous other scan sequences to be developed, enabling different image factors to predominate. For example, it is possible to suppress signals from fatty tissue or to highlight moving fluids to produce a magnetic resonance angiogram without the use of injected contrast materials.

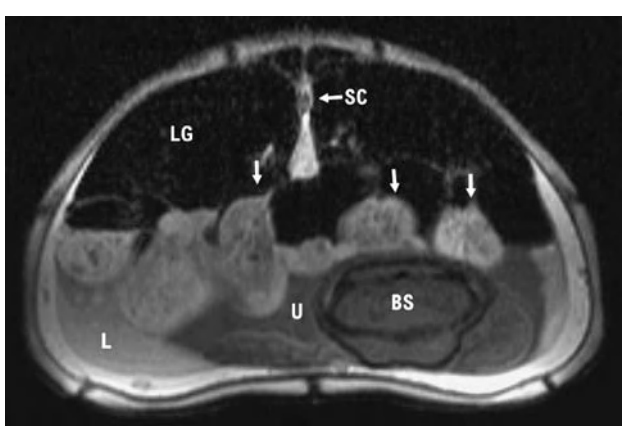

Fig. 8.98 T1-weighted transverse section of *Testudo graeca*, showing cross section of intestinal loops. Compare the appearance of distal colon cross-sections, marked with arrows, to the T2-weighted image in Fig. 8.99.
(Courtesy of Ian Calvert)

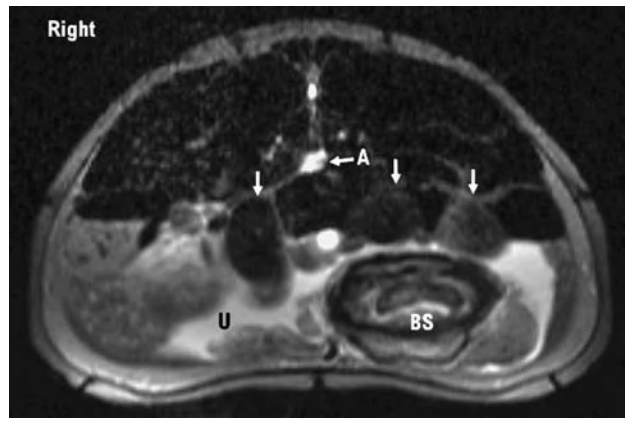

Fig. 8.99 T2-weighted transverse section of *Testudo graeca* at the same level as that shown in Fig. 8.99. Blood vessels appear as bright (white) on T2 scans.
(Courtesy of Ian Calvert)

Further image enhancement can be achieved with the use of intravenous injectable paramagnetic compounds. The most widely-used substance is gadolinium, a lanthanide metal, which is conjugated with diethylene triamine penta-acetic acid (DTPA) because the free metal is toxic (Gadian *et al.* 1985). Gadolinium has similar pharmacokinetic properties to iodinated contrast materials and is cleared by the kidneys in humans. It is commonly used to enhance contrast between tumours and the surrounding tissues. Several similar compounds are currently being investigated as contrast agents and monoclonal-antibody-tagged paramagnetic compounds are also being developed to provide contrast enhancement of specific tissues.

METAL OBJECTS

The magnets in an MRI machine are usually left on, and can produce a force up to thirty thousand times the Earth's gravitational pull. This force will also be applied to any magnetic metal object nearby or even within the patient. Metals are therefore hazardous objects in an MRI suite because of the possibility of them becoming airborne projectiles! Consequently it is important to plan an MRI scan on the basis that no metal objects will be used in the vicinity of the patient unless they are known to be non-magnetic. For the same reason, animals that have displaceable metallic material internally or orthopaedic metal work need to be assessed carefully prior to a scan since they could suffer injury or thermal burns if placed in an MR field. Permanently-fixed surgical stainless steel staples of the type used to seal blood vessels when spaying tortoises are usually safe, provided they have been in the animal long enough to be embedded in tissue (>8 weeks), however human aneurysm-type clips may move. It is unclear how microchips may react. Radiography is advised.

RESOLUTION AND AVAILABILITY

A major factor governing image resolution and scan speed is the magnetic field strength, which is why MRI magnets are both large and expensive. However, new miniature magnetic coils, using super conductor technology, are being developed to image smaller structures such as joints. These smaller machines are

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likely to be less expensive than whole-body scanning machines, and more widespread use may make this technology much more accessible in the near future. In the United Kingdom there is currently one dedicated veterinary mobile MRI scanning service, and some private human facilities are also willing to scan tortoises out of normal hours.

At the present time it is possible to achieve a resolution of 1–1.5 mm with human machines and even thinner slices have been imaged in reptiles with specially adapted nuclear magnetic resonance spectrometers (NMRS) (Anderson *et al.* 2000).

RESTRAINT

Compared to imaging techniques using high energy, low frequency radiation, acquisition of MRI images is a slow process—the scans used to produce the accompanying images took between 1.3–7 minutes. With the majority of chelonians this does not present major problems and with some animals it may be possible to obtain diagnostic images even without sedation. If necessary, restraint can be achieved by fastening the tortoise to a wooden board with Velcro strips.

However, it is sensible to insert a jugular catheter prior to the scan so that injectable anaesthetic agents such as alphaxalone/alphadolone acetate or propofol can be used if necessary. A pre-inserted catheter will also allow injection of contrast materials should these be required. The catheter should be fitted with an extension tube so that injections can be given without having to alter the animal's position.

Even with an anaesthetised animal, some motion artefacts due to breathing, gut peristalsis or ventricular contraction may degrade the image. Intestinal movement can be slowed or stopped by giving hyoscine/dipyrone (Buscopan compositum) at a dose rate of approximately 0.05–0.1 ml/kg IV (Figs 8.100–8.102).

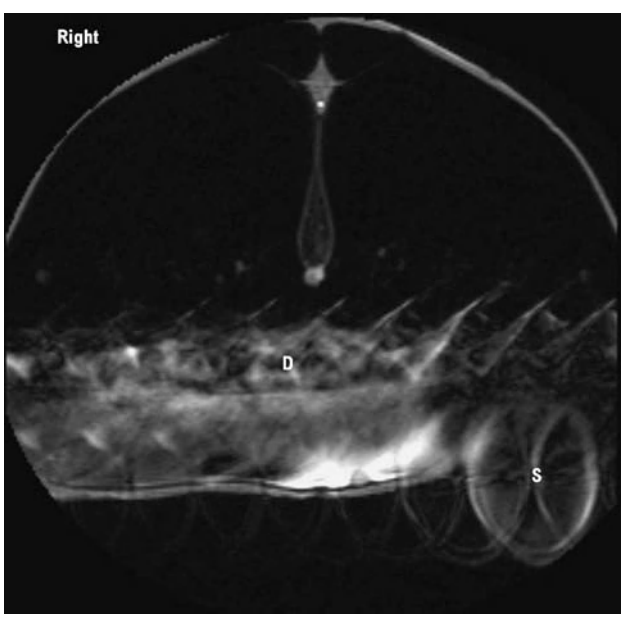

Fig. 8.100 T2-weighted transverse section of an Aldabran tortoise at the level of the duodenum and stomach, showing the marked movement artefact after introducing olive oil and cisapride via a pharyngostomy tube.
(Courtesy of Ian Calvert)

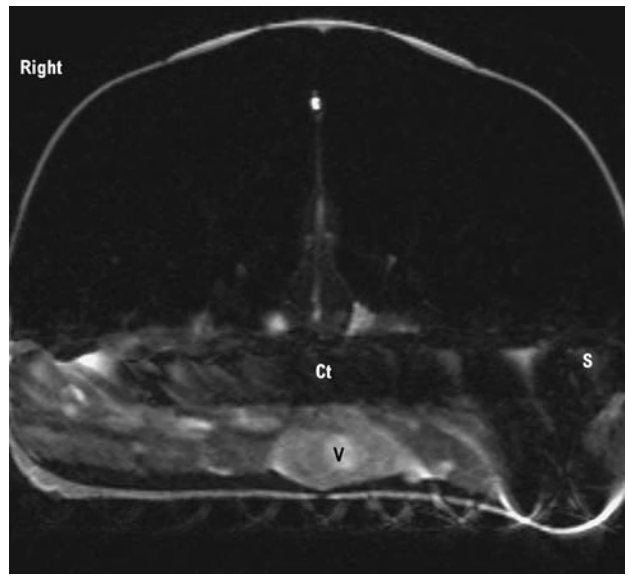

Fig. 8.101 T2-weighted transverse section of Aldabran tortoise, showing more moderate movement in the more cranial transverse colon. Both scans were done at the same time.
(Courtesy of Ian Calvert)

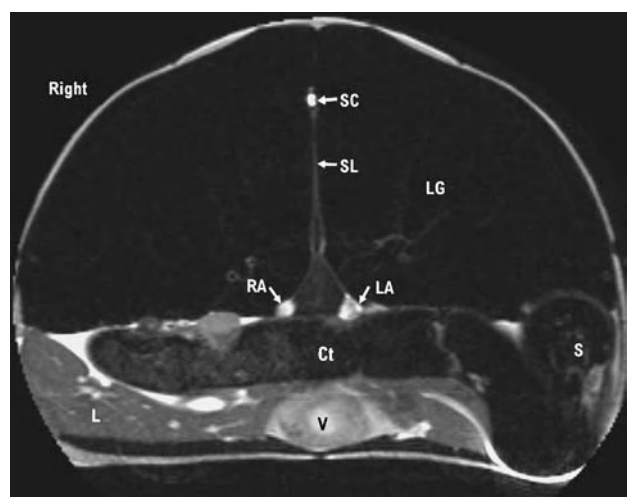

Fig. 8.102 T2-weighted transverse section of Aldabran tortoise at same level as in Fig. 8.101, but after an IV injection of Buscopan. Note the close proximity of the transverse colon to the pericardium.
(Courtesy of Ian Calvert)

VIEWS

It is possible to obtain scans (slices) in almost any orientation with MRI. However, standard coronal, transverse and sagittal slices are usually made, either of the whole animal or from the particular region of interest. This is one of the big advantages of MRI scanning; within a space of about 30 minutes it is possible to obtain sufficient images to build up a complete three dimensional appreciation of the animal's internal structure.

Cardiovascular structures

Recently, techniques have been developed that use electronic gating to acquire images during specific portions of the cardiac cycle. This allows heart chambers, valves and myocardial thickness

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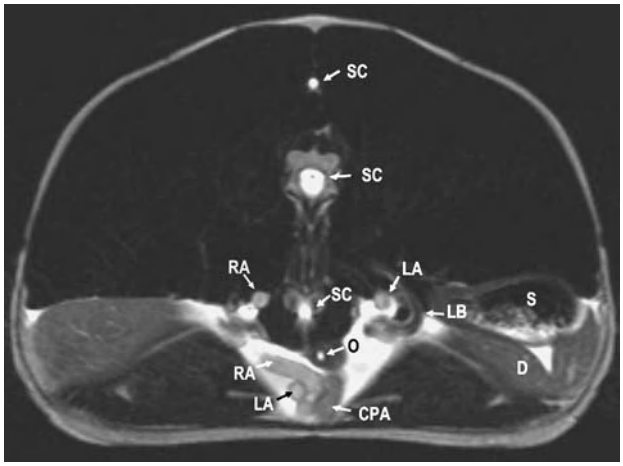

Fig. 8.103 T2-weighted transverse section of Aldabran tortoise. The great vessels can be seen at the base of the heart, the left bronchus can also be seen in section. Note the three cross-sections of the spinal cord due to the S-shaped curved neck when the head is withdrawn.
(Courtesy of Ian Calvert)

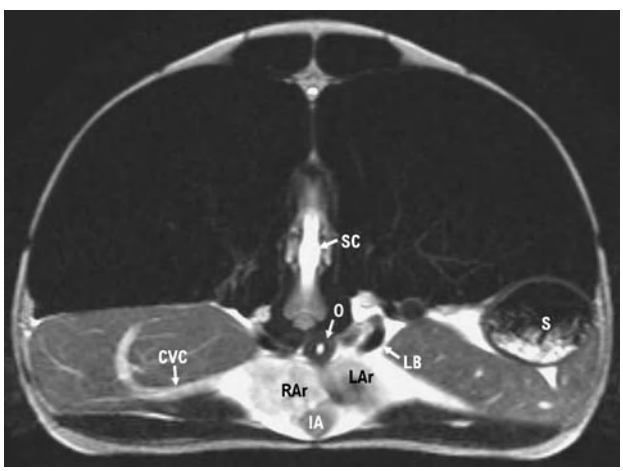

Fig. 8.104 T2-weighted transverse section of Aldabran tortoise. The caudal vena cava can be seen leaving the liver to join the sinus venosus. The left and right auricles and the innominate artery can also be easily distinguished. The oesophagus can be seen clearly because it contains a silicone pharyngostomy tube which contains olive oil.
(Courtesy of Ian Calvert)

and contractility to be observed with greater accuracy compared to ultrasound. Pericardial effusions and tumours in and around the great vessels and thyroid glands can now be easily imaged in humans, with the ability to scan in any plane (Hartnell 1991). This gives MRI a considerable advantage over ultrasound in assessing cardiac pathology in chelonians, where the carapace and plastron severely limit access with an ultrasound probe. Major blood vessels show up well and can be reasonably easily identified in most sections (Figs 8.103–8.105). Note that arteries containing oxygenated blood are less paramagnetic than veins and produce a greyer image than veins (see aorta in Fig. 8.105).

Lung fields

Chelonian lung fields can be seen clearly in MRI scans (Fig. 8.106) because the respiratory rate is so slow, and it is easy to appreciate the close apposition of stomach and kidneys to pleural tissue.

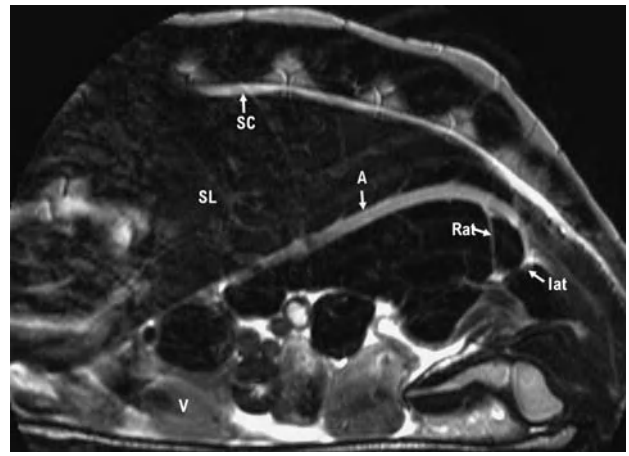

Fig. 8.105 T2-weighted sagittal section of Aldabran tortoise. The aorta can be seen arching caudally suspended from the carapace. A renal and an iliac artery can also be identified.
(Courtesy of Ian Calvert)

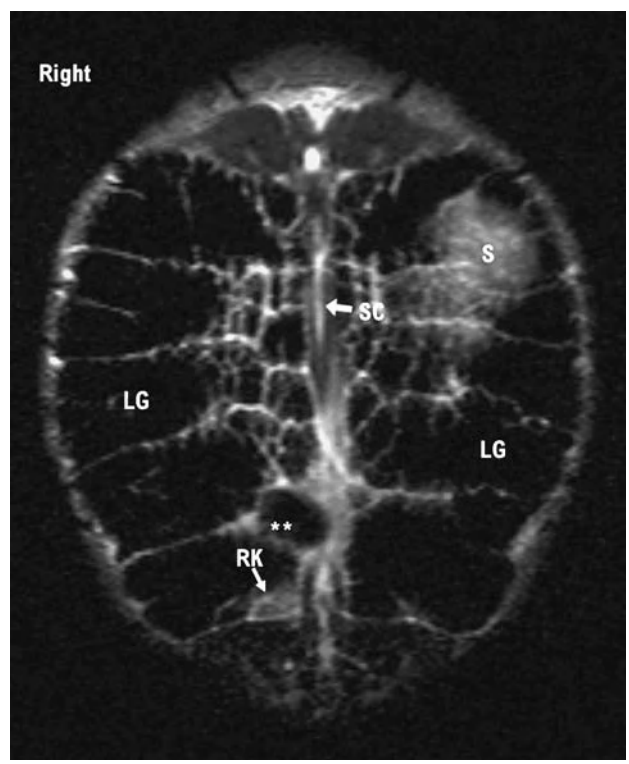

Fig. 8.106 T2-weighted coronal scan of *Testudo graeca*, showing lung fields. Note how the fundic region of the stomach ascends high above the other viscera and into the lung field. The site marked ** is where the distal duodenum turns and descends towards the plastron.
(Courtesy of Ian Calvert)

The pulmonary septae are clearly visible and inflamed pleural tissue shows up well on T2-weighted images (Rübel *et al.* 1994b). The trachea, its bifurcation and the main bronchi can also be readily assessed (Figs 8.103 & 8.107). Given the ability to image the lungs in all planes, MRI is a particularly sensitive way of evaluating and monitoring the extent of pulmonary disease in reptiles.

T1-weighted images have recently been used to locate pulmonary abscess/fibriscesses in a gopher tortoise (Pye *et al.* 1999).

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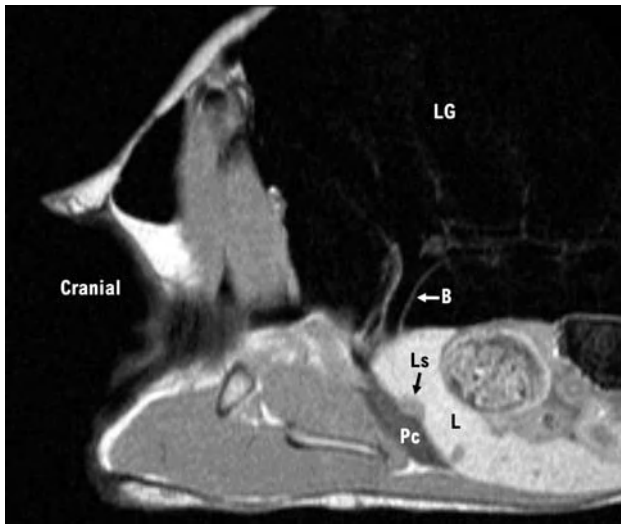

Fig. 8.107 T2-weighted sagittal section of Aldabran tortoise, showing the right primary bronchus and a small lesion in the liver.
(Courtesy of Ian Calvert)

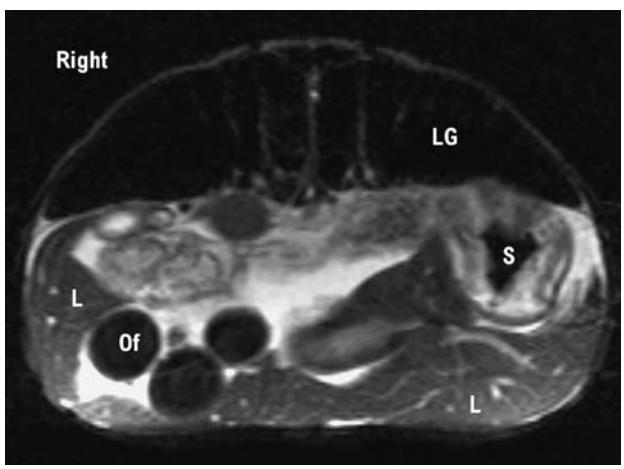

Fig. 8.108 T2-weighted transverse section of an ovulating *Testudo graeca*, showing various liver lobes and intrahepatic blood vessels.
(Courtesy of Ian Calvert)

Liver

The liver is easily distinguished on all views. T1-weightings show the parenchymal tissue well, whereas T2-weighted scans tend to highlight the vessels and fluid-filled structures, such as the gall bladder (Figs 8.108–8.110). Rübel *et al.* (1994b) report that the liver of tortoises with fatty liver syndrome is larger and is characterised by a decreased T2 signal. More recently, Kazumitsu (1999) has developed a 'flash sequence' MRI technique to quantify intrahepatic fat, so it is possible that MRI might provide a non-invasive way of assessing some types of liver pathology and monitoring improvements after treatment.

Intestinal tract

The intestinal tract is readily visible, and the ability to image the gut in several different planes is particularly valuable when trying to trace the course of the different sections of the intestines

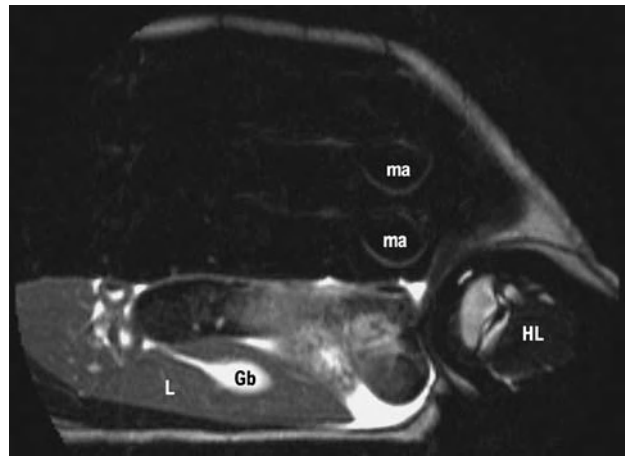

Fig. 8.109 T2-weighted right-sided saggital section of Aldabran tortoise showing the liver and gall bladder. A movement artefact can be seen just cranial to the right hind limb.
(Courtesy of Ian Calvert)

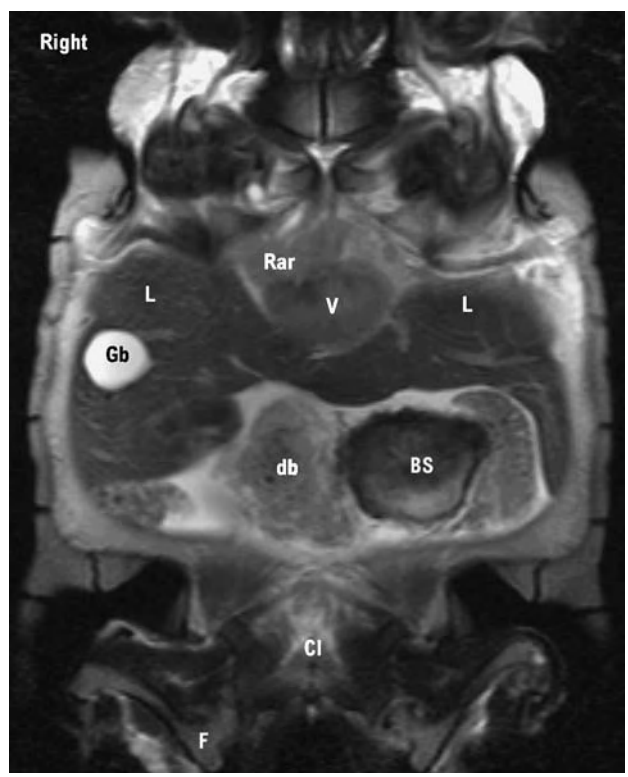

Fig. 8.110 T2-weighted coronal section of a *Testudo graeca*, showing heart, liver and a large bladder stone with adjacent debris in the bladder.
(Courtesy of Ian Calvert)

(Figs 8.111–8.112). Differences in water mobility enable dry, impacted faeces to be distinguished from softer, fresher faeces (Figs 8.98–8.99). From a research standpoint, MRI scans coupled with an appropriate marker compound would offer a very sensitive method of monitoring gut motility and assessing the effectiveness of motility enhancing agents in these animals.

Another method of visualising gut motility was discovered by the author while imaging an Aldabran tortoise (*Dipsochelys elephantina*) which had been fitted with an oesophagostomy tube with two small ball bearings embedded in its tip. These produced

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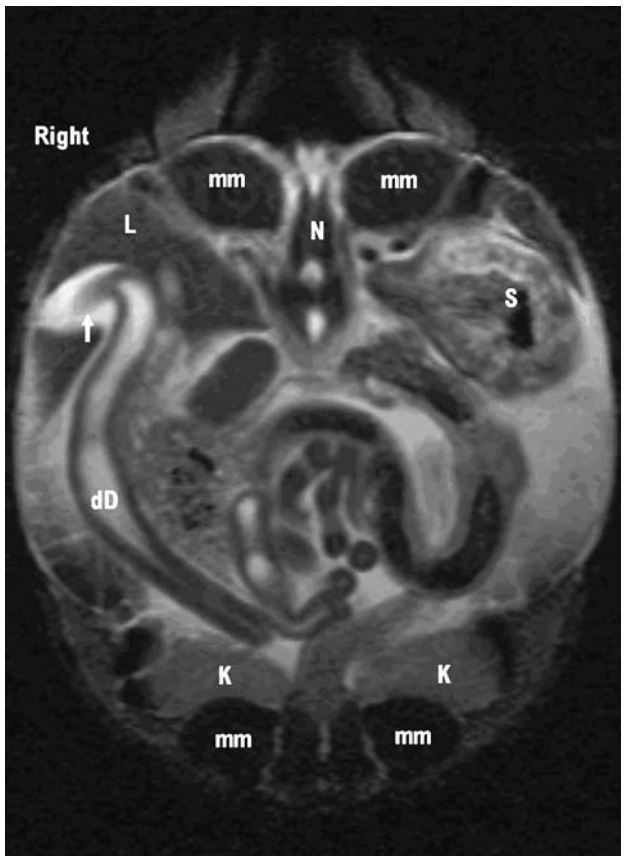

Fig. 8.111 T2-weighted coronal section of a *Testudo graeca* that shows the duodenum ascending sharply towards the dorsal surface (arrow), then travelling caudally along the right side of the animal over the rest of the viscera before plunging ventrally again alongside the right kidney. (Courtesy of Ian Calvert)

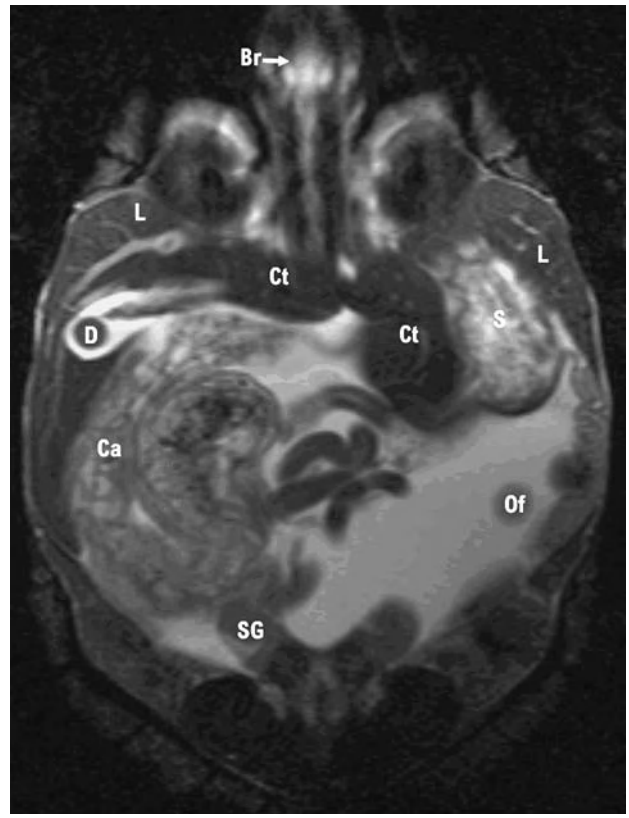

Fig. 8.112 T2-weighted coronal section of a hibernating *Testudo graeca*. This section is more ventral than that in Fig. 8.111, so the duodenum is seen only in cross section just behind the liver. The proximal duodenum and transverse colon cross the body in opposite directions with the duodenum being the more caudal. Their close association in this 'counter current' arrangement may aid in recycling water extracted from faeces in the colon through vascular arrangement. (Courtesy of Ian Calvert)

a considerable field artefact, which made intestinal movements after orally administered cisapride very obvious (Figs 8.101–8.104 & 8.113).

Raiti & Haramati (1997) obtained readily delineated, brightly-enhanced T1-weighted images of the intestinal tract in *Geochelone pardalis* by administering mineral oil at 6 ml/kg (total) over a two week period prior to scanning. (An oil-filled oesophagostomy tube can be seen as a bright circle in Fig. 8.104.) By introducing mineral or olive oil via silicon tubes per oesophagus or per cloaca it would be possible to highlight specific areas of the gastrointestinal tract between successive scans so that individual segments could be more easily identified.

Reproductive tract

Active, preovulatory follicles are very prominent features in MRI, and numbers, size and physical state of the follicles can be assessed (Rübel & Kuoni 1992) (Fig. 8.114). Differences between follicles, presumably in fat content, show up particularly clearly (Fig. 8.115), however inactive follicles are too small to be easily detected. Ovaries containing numerous follicles of ~2–3 mm diameter were present in the tortoise with the large bladder stone (Figs 8.98, 8.99) but are not easily identifiable.

Changes in calcification cannot be followed with MRI since calcified structures do not produce a signal. In one study, a

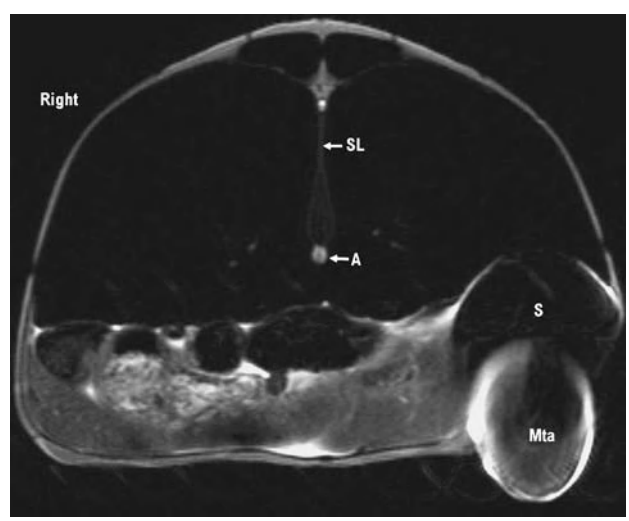

Fig. 8.113 T2-weighted transverse section of Aldabran tortoise, showing the effect of a small piece of metal on an MRI scan. In this case, there are two small, steel ball bearings embedded in the tip of a pharyngostomy tube which is in the stomach. The very strong field effect makes the stomach appear to be outside the plastron! (Courtesy of Ian Calvert)

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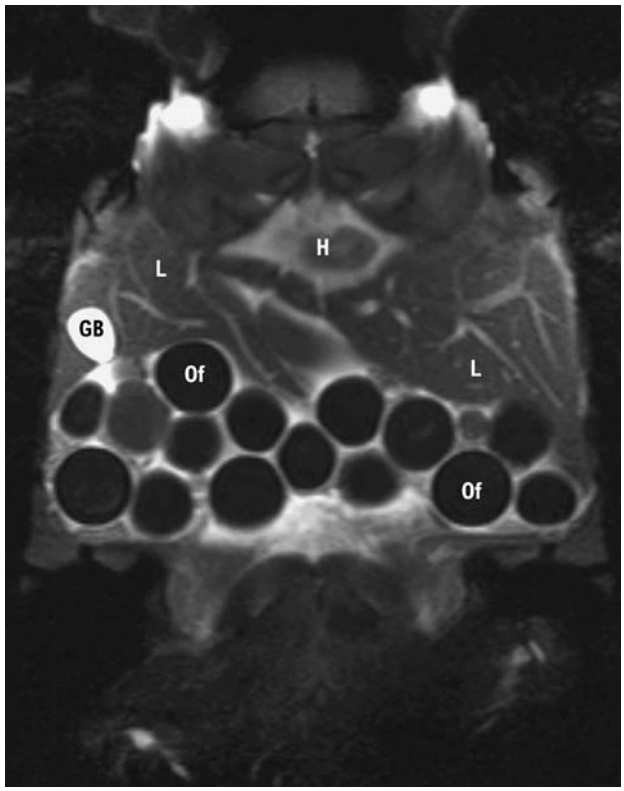

Fig. 8.114 T2-weighted coronal section of a hibernating *Testudo graeca*. Numerous developing ovarian follicles can easily be identified.
(Courtesy of Ian Calvert)

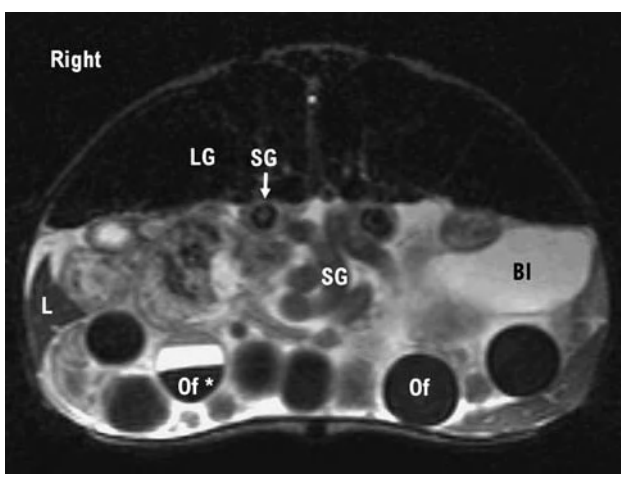

Fig. 8.115 T2-weighted transverse section of a hibernating *Testudo graeca* with ovarian follicles. One of these is clearly different from the others (*).
(Courtesy of Ian Calvert)

thickening of the shell-gland wall and a bright T2-weighted image suggested an inflammatory condition, which was subsequently confirmed by histology (Hartnell 1991).

KIDNEYS

Kidneys show up well and their size and position can be easily measured if T1- and T2-weighted images are obtained (Figs 8.116–8.119). The presence of hexamitiasis dramatically increased the size and coarseness of the MRI renal image (Rübel & Kuoni 1992;

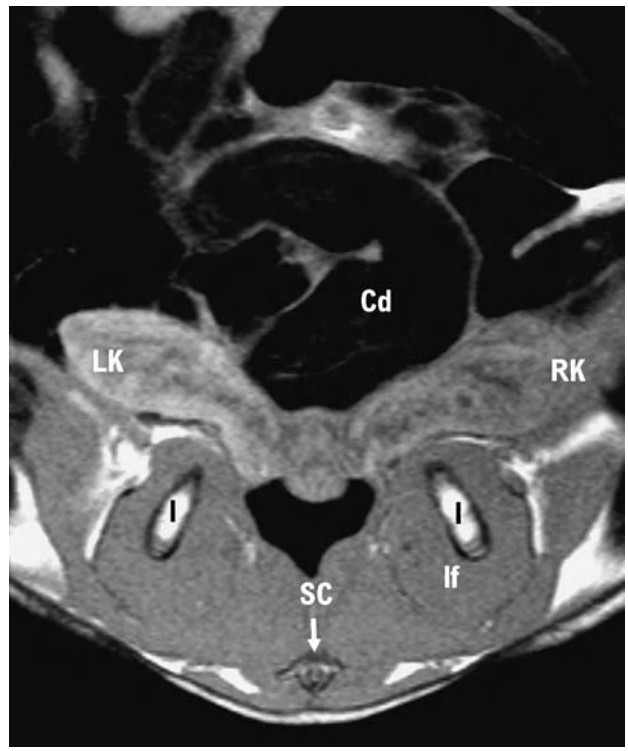

Fig. 8.116 T1-weighted coronal section of kidneys in an Aldabran tortoise.
(Courtesy of Ian Calvert)

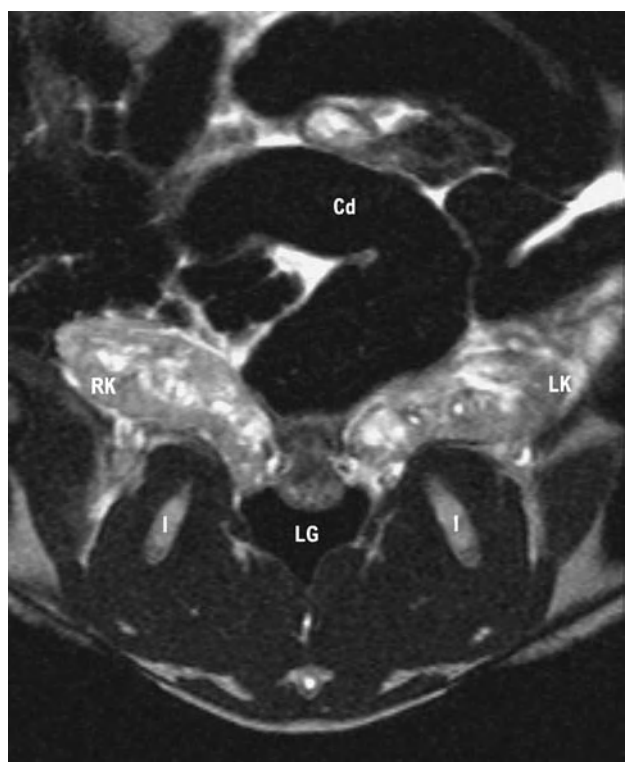

Fig. 8.117 T2 weighted coronal section of kidneys in the same Aldabran tortoise as Figure 20. The lighter areas presumably represent urine-filled collecting ducts leading to the renal pelvis and ureters. These kidneys look asymmetrical with the left kidney possibly being abnormal. Blood levels of uric acid, calcium and phosphate were within acceptable limits in this animal.
(Courtesy of Ian Calvert)

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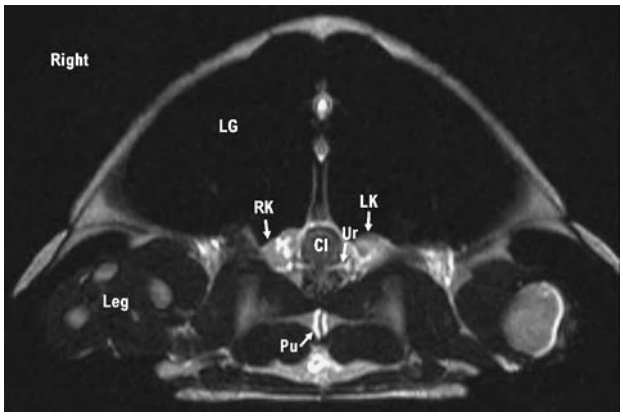

Fig. 8.118 T2-weighted transverse section of kidneys in an Aldabran tortoise. The ureters can be seen as two small bright tubes entering the cloaca.
(Courtesy of Ian Calvert)

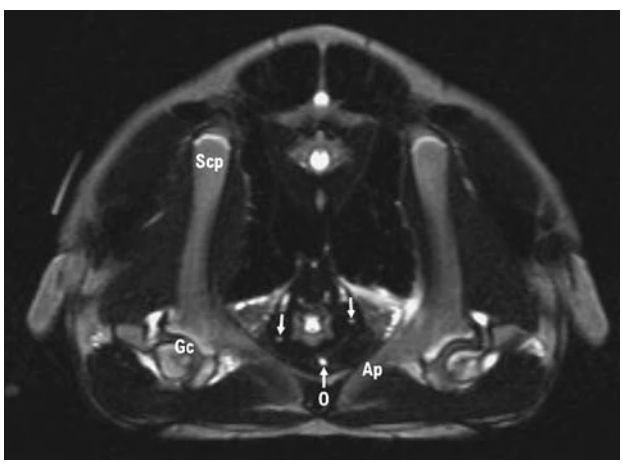

Fig. 8.119 T2 weighted scan through pectoral girdle of an Aldabran tortoise. Note the bright image of the pharyngostomy tube containing olive oil in the oesophagus and the left and right jugular veins (arrows) in the neck. The more ventral position of the right jugular is a consistent feature in other species.
(Courtesy of Ian Calvert)

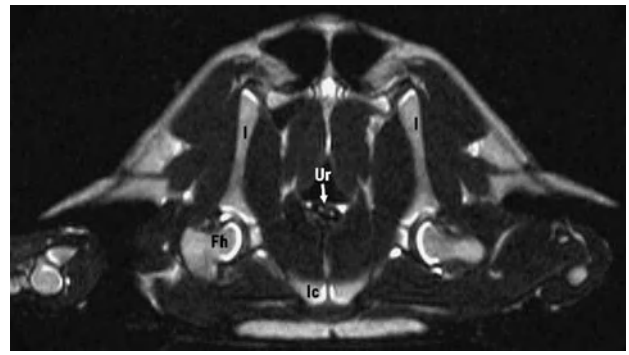

Fig. 8.120 T2-weighted scan through pelvic girdle of the tortoise in the previous figure.
(Courtesy of Ian Calvert)

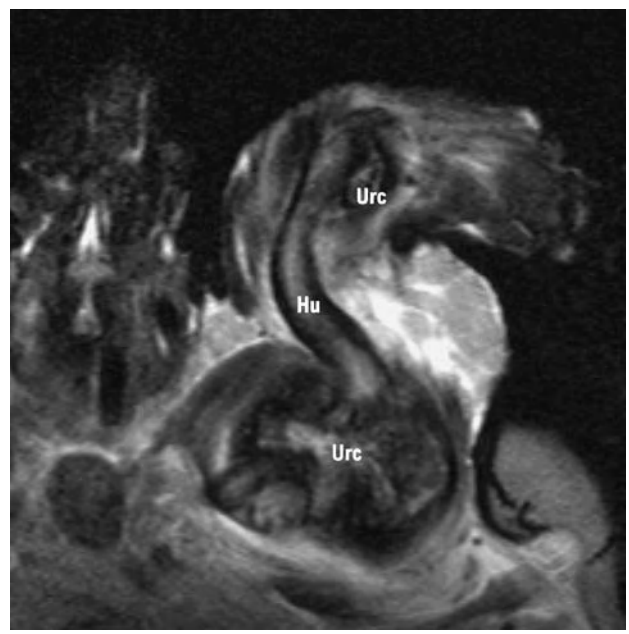

Fig. 8.121 T2-weighted scan showing gout in right foreleg of a *Testudo graeca*.
(Courtesy of Ian Calvert)

Rübel *et al.* 1994b) but so far no studies have been done to see if other causes of renal failure can be correlated with changes in kidney appearance.

Fluid within collecting ducts can be clearly visualised (Figs 8.117–8.118) and asymmetrical differences can be seen by comparing right and left kidneys. The short ureters can also be seen (Fig. 8.120).

Because MRI generates a digital image, considerable scope exists for computer image enhancement and comparison but such techniques have so far not been used with any reptile patient.

Bladder

The chelonian bladder wall does not image well on MRI, so it is difficult to determine if fluid is coelomic in origin or urine within the bladder (Fig. 8.99). If it becomes necessary to delineate the bladder, a paramagnetic fluid can be infused into the bladder via a catheter prior to the MRI scan. Although gadolinium would probably provide sufficient contrast it may be possible to use cheaper alternatives, such as a heparinised, deoxygenated blood sample or compounds containing ferritin. Deoxygenated blood is more strongly paramagnetic than its oxygenated counterpart and causes a decreased brightness in the T2-weighed signal (one reason why haemorrhage shows up well on MRI).

Despite their size, bladder stones can sometimes be difficult to visualise with conventional radiography but they show up well on MRI, and it is even possible to detect differences between individual layers within the stone (Figs 8.98–8.99).

Skeletal system

MRI is unable to image calcified tissues directly (bone cortices appear black on the MRI images in Figs 8.121–8.122), however bone marrow, cartilage, muscle, blood vessels and intra-articular fat all show up well, so that it is possible to detect subtle orthopaedic pathologies. MRI gives particularly useful views of the clavicular bones and pelvic girdle and the associated joints. In addition, urate deposits image well enabling the extent of gout to be assessed in joints and kidneys (Fig. 8.121).

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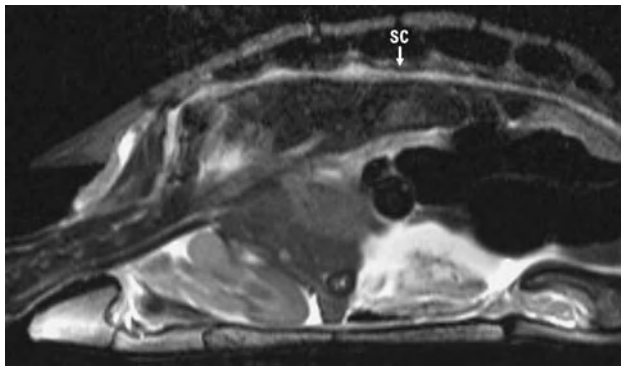

Fig. 8.122 T2-weighted saggital section of *Testudo graeca*, showing spinal cord.
(Courtesy of Ian Calvert)

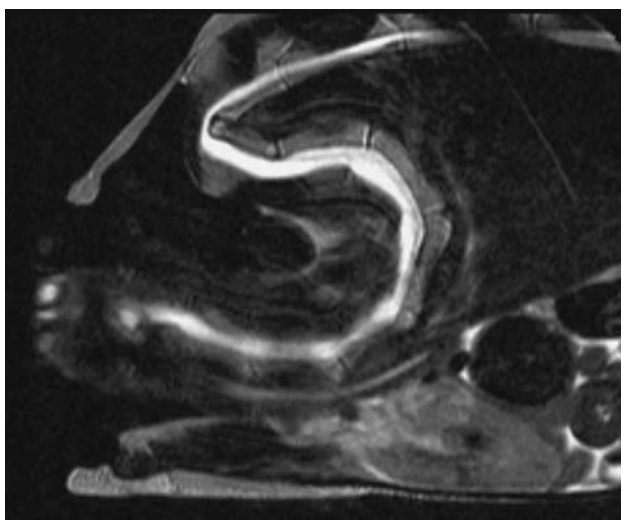

Fig. 8.123 T2-weighted saggital scan of an Aldabran Tortoise showing S-shaped nature of the cervical cord.
(Courtesy of Ian Calvert)

Nervous system

In humans, MRI was originally developed as a technique for imaging the nervous system, and this is the only non-invasive method of examining the chelonian spinal chord for evidence of trauma, infection or tumours. With careful orientation of the MRI 'slice', it is possible to image the entire length of the rather thin spinal chord (Fig. 8.122). The unique curvature of the chelonian cervical spine can also be readily appreciated (Fig. 8.123). Excellent images of the brain and eyes can also be obtained. Some idea of the potential resolution can be seen in a recent study where MRI was used to visualize structures within the brain of garter snakes (Anderson *et al.* 2000).

COMPUTED TOMOGRAPHY (CT)

(Michaela Gumpenberger)

CT is a radiography-based, non-invasive, cross-sectional, diagnostic imaging technique that offers significant advantages for detection of pathology in chelonians (Figs 8.124–8.133).

The scans are produced by a radiographic source and detectors rotating around the patient. When X-ray photons pass through

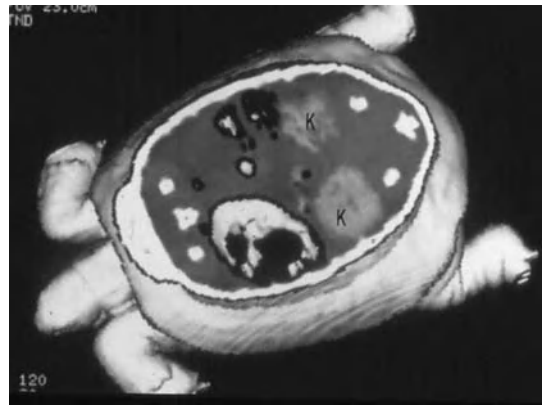

Fig. 8.124 *Testudo hermanni:* three-dimensional reconstruction of a male tortoise with hypercalcaemic kidneys (K).
(Courtesy of Michaela Gumpenberger, University of Vienna)

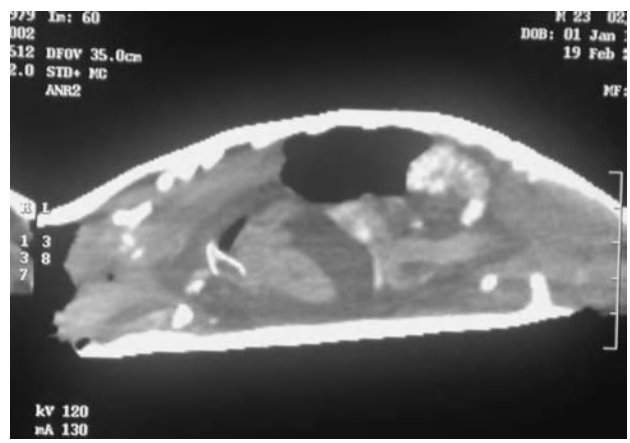

Fig. 8.125 Long-necked turtle: slightly paramedian sagittal CT scan of a long necked turtle with hyperuricaemic kidneys (gout) and pericardiac effusion (soft-tissue window).
(Courtesy of Michaela Gumpenberger, University of Vienna)

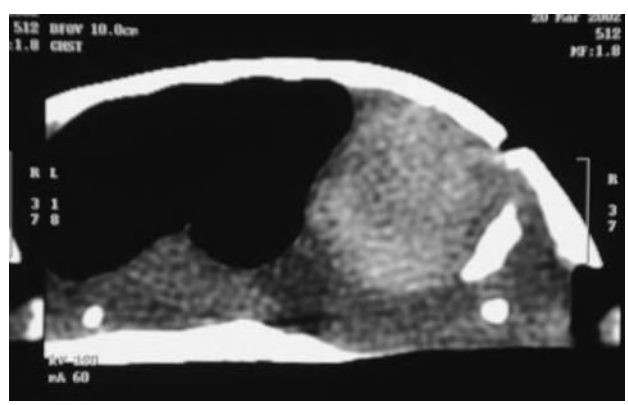

Fig. 8.126 Musk turtle (*Kinosteron* sp.): paramedian, sagittal CT scan of a musk turtle in soft-tissue window. A huge, hyperdense abscess, originating from shell necrosis, may be seen.
(Courtesy of Michaela Gumpenberger, University of Vienna)

tissues the absorption coefficients are measured and converted into individual grey shades. Therefore tissues can be identified by their grey shades or absorption coefficient numbers. This densitometry is expressed in Hounsfield Units (HU) (Bourdeau *et al.* 1992).

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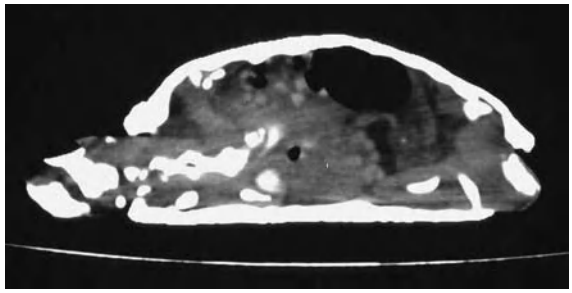

Fig. 8.127 Red-eared slider: slightly paramedian sagittal CT scan of a slider (soft-tissue window) suffering from multiple, small, hyperdense abscesses in the cranial lung and mid-coelomic cavity.
(Courtesy of Michaela Gumpenberger, University of Vienna)

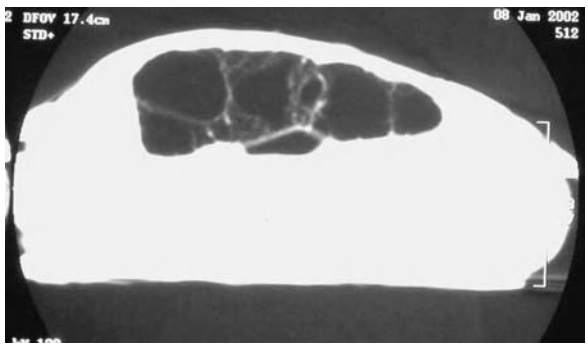

Fig. 8.128 Red-eared slider: slightly paramedian sagittal CT scan of a slider (lung window) suffering from pneumonia. The normal reticular lung pattern is missing.
(Courtesy of Michaela Gumpenberger, University of Vienna)

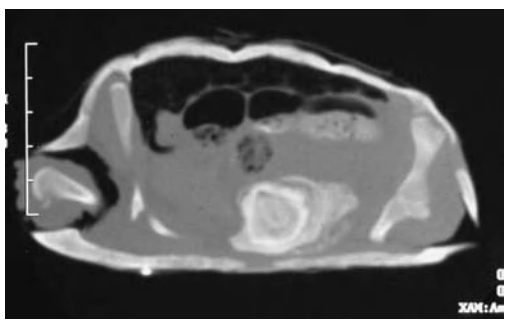

Fig. 8.129 African spurred tortoise (*Geochelone sulcata*): paramedian (at the level of the left hip joint), sagittal CT scan in bony window. A urinary bladder stone is visible.
(Courtesy of Michaela Gumpenberger, University of Vienna)

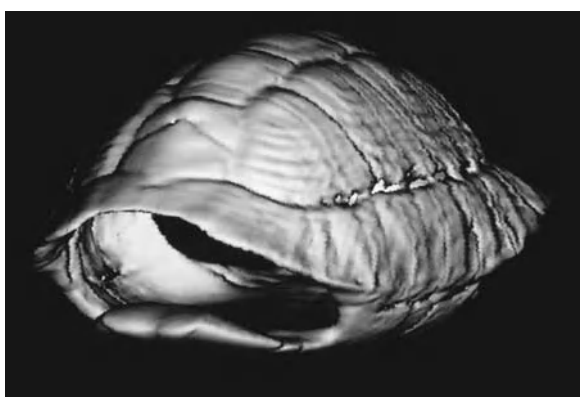

Fig. 8.130 Testudo hermanni – shell 3D (normal): three-dimensional reconstruction of the shell of a wild-caught male Hermann's tortoise with normal mineralisation of its shell.
(Courtesy of Michaela Gumpenberger, University of Vienna)

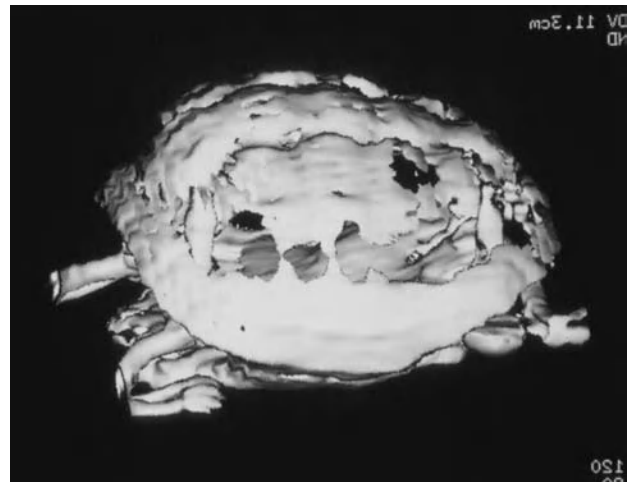

Fig. 8.131 *Testudo hermanni* – shell in 3D (*hyperparathyroidism*): three-dimensional reconstruction of the shell of a juvenile male Hermann's tortoise suffering from secondary hyperparathyroidism (note the bone loss of the carapace in comparison to the normal male in Fig. 8.130). Normal patterns of shell development are yet to be determined using CT.
(Courtesy of Michaela Gumpenberger, University of Vienna)

Fig. 8.132 *Testudo hermanni*: paramedian sagittal CT scan of a Hermann's tortoise (soft-tissue window). In the mid coelomic cavity is a fully-calcified but deformed egg. This animal did not suffer from dystocia, but produced a normal clutch with one broken egg.
(Courtesy of Michaela Gumpenberger, University of Vienna)

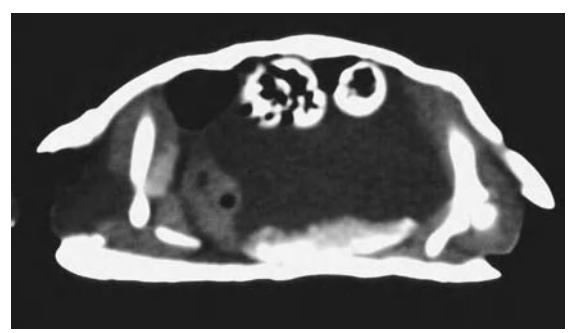

Fig. 8.133 *Testudo hermanni* – urine retention: paramedian sagittal CT scan of a Hermann's tortoise (soft-tissue window). The urinary bladder is enlarged and contains some deposits (hyperdense structures). Because of the dilated urinary bladder, the bowels are displaced dorsally.
(Courtesy of Michaela Gumpenberger, University of Vienna)

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Fig 8.134 Restraint for CT used by the author [MG].

In order to prevent movement blur, chelonians are fixed onto wooden blocks, placed in boxes or the patient's legs are taped into the shell (Fig. 8.134). Usually there is no need for anaesthesia. Most examinations are performed in sagittal planes with 1–5 mm thick consecutive slices. Additionally it is possible to reconstruct the other axial and horizontal planes as well as three-dimensional models. A whole examination (including positioning the animal) will last 3–5 minutes.

In comparison to conventional radiographic examinations there is no superimposition of structures. A lot of information is obtained regarding the skeleton, liver, gall bladder, urogenital, gastrointestinal and respiratory tracts in CT. This non-invasive technique gives additional help for planning surgery, quick follow-up examinations or teaching (Gumpenberger *et al.* 2001) (Table 8.7).

Table 8.7 CT scan views.

Sagittal scans	The sagittal scan, made in ventral recumbency, appears to be the most practical examination plane for smaller tortoises or turtles. It is similar to lateral radiographs and allows evaluation of most of the inner organs. Sagittal examinations require fewer slices than axial scans and therefore examination time and radiation exposure can be less.
Transverse scans	Transverse scans are commonly used for CT examinations in larger individuals. Symmetrically positioned organs can be compared directly.
Horizontal scans	Horizontal reformatted images are similar to common dorsoventral radiographs. It is unnecessary to fix the animal in lateral recumbency, which would cause displacement of the internal organs. The ventral lung fields can be compressed by abdominal organs and this could be misinterpreted as lung pathology.

Table 8.8 summarises the uses of CT scans.

In summary, CT is easier to learn and quicker to perform than MRI. There are few artefacts caused by ventricular contraction, breathing or gut peristalsis. However, MRI studies give much better soft-tissue detail.

Table 8.8 The uses of CT scanning.

Shell and skeleton	Size, shape, structure and density of the shell and skeleton are well illustrated by CT scans (Abou-Madi 2001; Raiti & Haramati 1997; Rübel *et al.* 1994b). CT is the diagnostic tool of choice for examining skeletal disorders and is therefore complementary to MRI studies. Fractures, luxations, arthritis, necrosis (Fig. 8.126) and demineralisation (Figs 8.130–8.131) can be detected easily. Demineralisation can be proved by densitometry: e.g. normal compacta of the shell of Mediterranean tortoises measures 950–1300 HU (Hounsfield units); in chelonians with secondary hyperparathyroidism, compacta measures 350–550 HU.
Stomach and intestines	The gas-filled stomach is easy to find. Bowel loops are hard to differentiate when normal (Rübel *et al.* 1994b). Contrast media can be used for better visualisation (Meyer 1996).
Liver	Enlargement of the liver can be detected (mainly in horizontal reformatted scans). Densitometry of the liver helps in the diagnosis of fatty-liver disease (decrease from 50–70 HU to –10– –40 HU) or dystrophic calcifications.
Female reproductive tract	CT scans give detailed information about number, size, shape, density and position of follicles and eggs (Gumpenberger & Hittmair 1997) (Fig. 8.132). While ultrasonography is used to monitor egg development, it is impossible to count the definite number of follicles because of other overlying follicles or eggs.
Urinary tract	In most patients, the kidneys are found easily, especially when enlarged (Fig. 8.126). Gout often causes increasing density of the kidneys (Figs 8.124–8.125). Urine retention (Fig. 8.133) or urate deposits (Fig. 8.129) in the urinary bladder are easily visualised.
Respiratory tract	Slow respiratory rates permit high-quality scans of the typical reticular pattern and specific pathological findings of the chelonian lung. In combination with densitometry, it is easy to detect haematomas, pneumonia, abscesses (Fig. 8.128). There are no overlying structures, e.g. the shell or digestive tract, as in conventional radiographs.
Heart	Position, size and shape can be differentiated as well as the ventricles (Fig. 8.125).
Space-occupying lesions	Parenchymal texture and origin of abscesses and tumours can be detected (Fig. 8.126).

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SCINTIGRAPHIC IMAGING

Following placement of an intravenous jugular catheter, Hernandez-Divers *et al.* (2002) gave 99 m (sic) Tc-methelyne diphosphonate (MDP®, Athens Isotopes, Athens, GA) intravenously to a Horsfield's tortoise (*Testudo horsfieldi*) and performed three-phase bone scintigraphy using an Omega 500 gamma camera® (Technicare, Solon, OH) and a computer.

The authors found that it was easier to visualise a diseased plastron in this animal using scintigraphy when compared to radiography and they suggest the technique may be of use in the investigation of bone lesions (Figs 8.135–8.136).

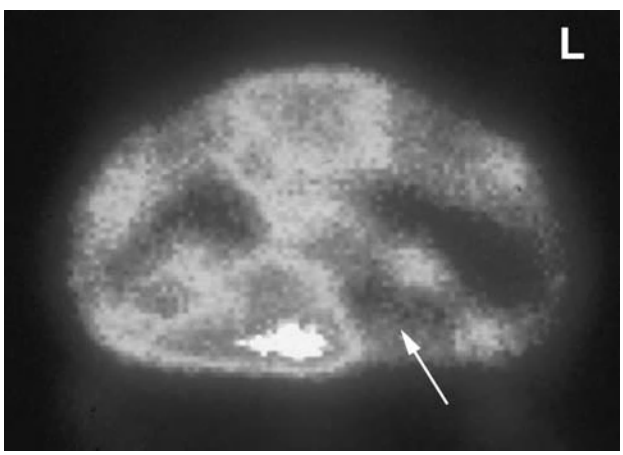

Fig. 8.135 Cranio-caudal view of a scintigraph of a *Testudo horsfieldi* with plastron necrosis. Note that the areas of photopaenia (arrows) correspond to bone necrosis.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

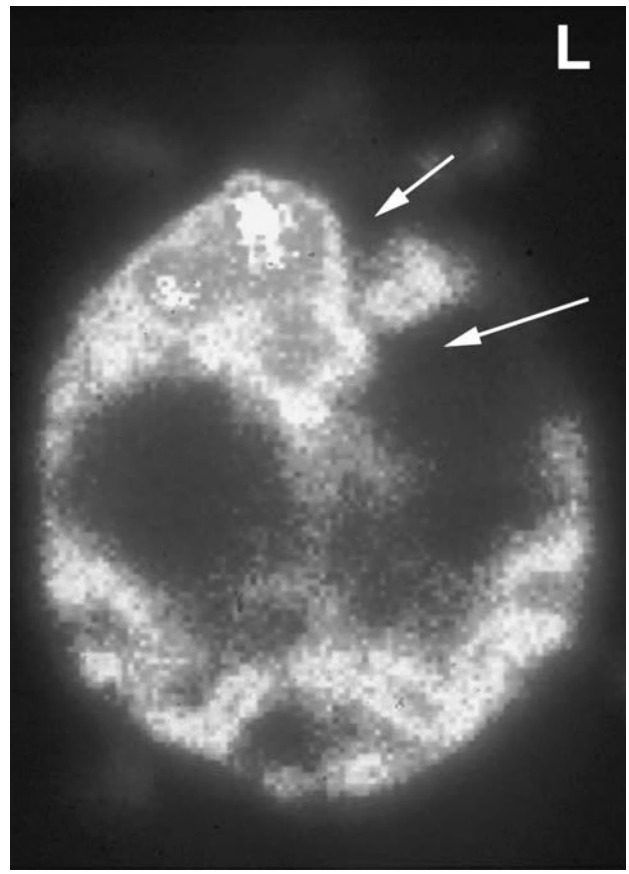

Fig. 8.136 Dorsoventral view of a scintigraph from the *Testudo horsfieldi* in Fig 8.135. Again the photopaenic area (arrow) corresponds to bone necrosis.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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HOSPITALISATION

STUART MCARTHUR

Hospitalisation is the admission of a patient into a diagnostic or therapeutic environment with a view to improving its health.

It is essential for those hospitalising tortoises to have a basic knowledge of the habitat type and seasonal variations preferred by the species they are keeping. There should be access to a database of reptile husbandry information as there are profound differences in the requirements of different species. The conditions in which captive chelonians have been maintained in the period prior to their presentation with illness are often directly linked to the illness itself. In fact they are commonly the causes. At this author's (SM) surgery we often appear to 'cure' sick chelonians simply by correcting inadequate husbandry and nutrition.

Conditions suitable for the hospitalisation of sick chelonians should promote an environment suitable for patient recovery. Therefore adjustments in humidity, light and temperature-provision should be made, in order to maximise patient benefit and recovery. Consideration should be given to factors such as the typical wild environment of the species: heat provision and thermoperiodicity, photoperiod and quality of light, humidity provision, socialisation (if appropriate), environmental enrichment (if appropriate), access to bathing and water sources and nutrition. Additional preparations and care may be required where a hibernating or aestivating species is being nursed.

Klingenberg (1996a) assessed the relative merits of hospitalising sick reptiles as an alternative to out-patient care. At our clinic, we share Klingenberg's view that reptiles requiring anything more than very basic home treatment benefit from initial veterinary stabilisation. Stabilisation is achieved through temporary hospitalisation in an appropriate environment with appropriate standards of care. Most sick chelonians presented at the author's surgery are hospitalised for 7–14 days during initial stabilisation. Many chronically-ill chelonians are hospitalised for several months.

Jarchow (1988) produced the first fully-comprehensive guide to the hospitalisation of sick reptiles, and little similar work has been published since, although recently a short roundtable discussion has appeared in the *Journal of Herpetological Medicine and Surgery*.

BENEFITS OF HOSPITALISATION

Tortoises and turtles often suffer from chronic diseases demanding specialised nursing care during recovery. Chelonians are less easy to medicate at home than mammalian patients and the veterinarian remains responsible should a problem arise with either patient or owner as a result of home treatment. In cases of chronic illness general supportive care is of the greatest importance. Fulfilling fluid, environmental and nutritional requirements can be guaranteed to a hospitalised patient. If an animal is to be cared for at home then a written care plan, covering all aspects of environmental provision, nutrition, nursing, drug storage, dose and administration technique, is essential.

At our clinic we believe that stabilisation before anaesthesia and surgical procedures, and subsequent peri-operative management of surgical patients, is best carried out in a hospital environment. Care may then be passed on to a keeper on an outpatient basis, when they are medically capable of dealing with patient care and assessment. The keeper's abilities and experience, as well as the clinician's, are important. Some keepers will provide comparable care to that of a reptile clinic, allowing early release to their care.

It is unlikely, however, that most keepers will meet the stringent requirements of a sick reptile in the immediate post-consultation period. Most conditions presented at the author's clinic have arisen as a direct consequence of the keeper failing to understand the requirements of the chelonian.

Diagnosis

Hospitalisation allows accurate observation over time under controlled environmental conditions. It maximises the ability of a clinician to assess patient health allowing the assessment of physical signs not apparent in a 10–30 minute consultation. (There is no substitute for the ability to observe the spontaneous behaviour of a hospitalised patient. It is unlikely that an animal will behave normally whilst on the examination table.)

As a consequence of transportation, few chelonians are presented at our surgery with core temperature within their appropriate temperature zone (ATR). During diagnostic hospitalisation, core temperature can be elevated to within a chelonian's ATR. Behaviour, physical strength, appetite and other functions indicating the general condition of the patient may be dramatically different with the reptile at or near its preferred body temperature (PBT) as compared to the temperature when presented for examination.

Hospitalisation also allows the removal of many external influences detrimental to the animal's health. It therefore helps to reveal the extent to which internal factors are involved in disease. Many diseases of reptiles are primarily external (exogenous or environmental) and only become significantly internal (or endogenous) with time.

Stabilisation

Hospitalisation allows the correct therapeutic environment to be provided at once. Although owners can be advised of ideal husbandry, it would be unusual in our experience (SM/RJW) for all problems to be rectified at home without a period of trial and error. If deficiencies in management are frequently factors in the

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onset of disease then it is unlikely that the more stringent requirements of the sick reptile will be met in the immediate post-consultation period.

Hospitalisation allows fluid therapy, medication and nutritional protocols to be followed in a controlled environment. It may take 10 or more days of fluid administration, for instance, to re-establish renal function and produce urination in a profoundly dysuric and dehydrated, post-hibernation animal. This is plainly better accomplished in a hospital environment.

Patient monitoring

A continuous opportunity for the observation of changing clinical signs is important. Clients who must travel large distances to a referral centre will not be able to return for frequent follow-up checks in order to monitor an animal's progress. In addition, repeated transportation backwards and forwards to a veterinary surgery is unlikely to assist the recovery of the chelonian, and will also complicate clinical assessment.

Accurate monitoring of the patient is possible and can be recorded on appropriate hospitalisation forms (recording daily weight, fluid intake, urine and faecal output and quality, appetite, demeanour, etc.). Pathological and diagnostic samples (blood, faeces, urine, oral swabs) can be collected as needed, or as availability or inspiration arises. Follow-up samples may allow progress to be assessed over time.

Pain control

Adequate pain control would generally involve regular dosing with prescription-only analgesics by injection, and therefore cannot be carried out at home by a non-veterinarian client.

Complex management/therapy

Hospitalisation permits therapy not possible at home. Examples of this are complex surgery or prolonged fluid therapy.

Hospitalising a patient provides an invaluable opportunity for continual reassessment while different therapeutic options and ideas are explored. It is also prudent to hospitalise those chelonians that have been given a very guarded prognosis. These animals can then be euthanased if deterioration and distress are apparent, without the possibility of a difficult emergency call-out involving the keeper's making critical health and welfare assessments.

Chelonians frequently require ongoing drug therapy but are less easy to medicate at home than conventional mammalian pets. Whilst the placement of semi-permanent oesophagostomy tubes can facilitate enteric drug administration at home, many drugs must still be administered by injection. Although some owners become competent with injection and other techniques, it should not be forgotten that the vet remains ultimately responsible should a problem arise with either patient or owner as a result of the treatment protocol.

Medium- to long-term care

Hospitalisation allows the patient's condition and bodily functions to be assessed, in order to formulate a care plan for the individual as an outpatient. Upon discharge into the keeper's care it is possible to advise the keeper of the criteria that would suggest that representation to the veterinarian is required (e.g. inadequate frequency of urination or bowel movement, lack of appetite or activity, weight loss).

Rehabilitation of wild species

Wild species require stabilisation and careful assessment of future viability prior to release. Jacobson *et al.* (1999a) provide an excellent decision-making tree that helps us manage wild chelonians. There is no benefit in returning to the wild chelonians that are unable to fend for themselves, are weak or underweight. These animals require appropriate stabilisation.

PROBLEMS ASSOCIATED WITH HOSPITALISATION

Size

Large species (>45 kg) have the strength to damage themselves and treatment facilities. Such individuals may be better accommodated in enclosures at home, and the veterinarian should consider visiting.

Cost

The cost of providing an appropriate nursing vivarium suited to the species in question must be recovered in private practice through appropriate charges to the client. When quoted a suitable charge, the client may decline it. In order to provide a similar level of patient care as the hospital environment, the client will then need copious advice on how a comparable system can be set up at home.

This negotiation may take up significant veterinary or nursing time. It is important to point out the advantages of hospitalisation described earlier. Ultimately, at the author's clinic, most keepers can be persuaded that recovery is more likely if the animal is temporarily hospitalised.

Separation anxiety

Many clients develop anthropomorphic attitudes towards their animals. They may believe that separation from their chelonian will be detrimental to its condition. This is seldom so, in our experience. Such clients can be encouraged to visit the reptile regularly.

Inadequate hospitalisation and maladaptation

It is necessary to research and provide the best care possible. By confining an animal we prevent that animal and its keeper from managing its own day-to-day requirements. Inappropriate hospitalisation will have a serious destabilising influence upon reptile health. Failure to provide fluids, nutrition, temperature, light and humidity suited to the animal being cared for may result in maladaptation (and may leave room for future litigation).

Jarchow (1988) suggests the stressors involved in hospitalising reptiles include:

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- malnutrition;
- inappropriate ambient temperature;
- inappropriate humidity;
- inappropriate light provision or photoperiod;
- inadequate cage size;
- the lack of hiding places or essential cage appliances;
- insufficient thermal gradients within the cage;
- inadequate water provision;
- intimidation by cage mates;
- excessive handling or disturbance by humans.

Maladaptation as a result of relocation to the hospital environment is unusual where the clinician and nursing staff are appropriately experienced and skilled. Appropriate patient observation, assessment and adjustments in environmental provision should aim to provide a sick reptile with conditions suited to recovery.

Jarchow (1988) suggests that a maladapted reptile may be more susceptible to infectious and other diseases. They may display anorexia, weight loss in spite of regular feeding, reproductive failure, excessive torpor and persistent escape-type behaviour. This compromises patient assessment and recovery.

PRE-TREATMENT ASSESSMENT

At admission, details of fluid intake (before and after hibernation), frequency and quality of urine and faecal output and last observation of these, appetite, activity, environment provision (light, heat, bathing, etc.) must all be recorded as part of the anamnesis. In terrestrial species, understanding the response to fluids and other therapies over time is important to case management. It is important to be able to gauge how things have changed after admission. Weight should be recorded and a pre-treatment blood biochemistry assessment is strongly advised, taken from a jugular vein if possible.

ACCOMMODATION

This author regards most cat and dog hospitalisation accommodation as unsuitable for housing reptiles unless exceptional circumstances, such as patient size, necessitate their adaptation and conversion. It is otherwise inappropriate to hospitalise reptiles without some form of vivarium. Nursing vivaria differ from enclosures used to maintain captive reptiles, as disease control and recovery are primary considerations (Figs 9.1–9.28). They must either be disposed of after use, or adequately disinfected before a new patient is hospitalised. This means that vivarium furniture and aesthetic designs are generally reduced.

The materials needed to construct such a hospital enclosure are variable, depending on life expectancy and the number of animals likely to be hospitalised within the enclosure during its life. Vivaria should be constructed out of non-porous, non-abrasive, disinfectable materials e.g. plastic, glass, Formica/MDF, painted or sealed wood or steel with sealed joints and edges. Suitable examples include a purpose-built plastic and steel vivarium or an open-topped plastic, melamine or glass tank/box. Cat and dog cages are unsuitable for housing reptiles unless exceptional circumstances, such as patient size, necessitate conversion.

Requirements for large, freshwater and marine species differ greatly from small, semi-aquatic and terrestrial specimens. Further advice regarding these animals is given later.

Fig. 9.1 Reptile wards benefit from having a selection of different vivaria set-ups. Here four types of chelonian accommodation are displayed.

Top right is a glass tank vivarium. On the left side of this tank is a warm basking area and to the right is a cooler and more humid area with UVB exposure, humidity provision (note the tank condensation) and hides. To the left are two large vivaria suited to large basking species such as *Geochelone pardalis*. Bottom right are two versatile hospitalisation tubs. One has been set up to dry dock a semi-aquatic animal (illustrated). The other is clean and ready to use.

Fig. 9.2 Facilities to house sick chelonians should be constructed of materials such as plastics, which are easily disinfected.

Each animal should have unique items for feeding, and hides, bathing trays, medicine containers and anything else which may come into contact with the animal should be easily wiped clean.

Spread of airborne pathogens seems unusual in chelonians so the use of a common airspace remains possible under close supervision and monitoring. The use of sentinel animals will help to indicate if disease control techniques are adequate.

GENERAL POINTS

- It should always be possible to disinfect veterinary treatment enclosures and isolate potential infectious agents.
- Ventilation and disinfection are primary construction considerations. Open tops are preferred for most chelonians.
- It should be possible to monitor and control temperature, light and humidity.
- Substrate may need careful selection to avoid problems associated with fire risks, ingestion, wound management or clinical-waste management (this author uses chipped hemp bark). Substrates commonly used for tortoises include alfalfa/grass pellets, bark chippings, hemp substrates, newspaper, shredded paper, Astroturf, indoor/outdoor carpeting and peat/soil mixtures. Sand, cat litter, and crushed corn cob or walnut shells are not recommended due to the risk of ingestion and intestinal blockage.

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Fig. 9.3 Basking chelonians benefit from direct exposure to ultraviolet light sources. Animals at this author's surgery (SM) behave very differently if maintained at similar temperature with and without ultraviolet exposure. Ultraviolet and basking lamps should be controlled so that the photoperiod is known.

Open-topped vivaria are preferred for housing basking species, as ventilation is improved and respiratory disease decreased.

Fig. 9.4 A typical hospitalisation tank for a basking species such as *Testudo horsfieldi* at the author's surgery (SM).

In these tanks both basking spotlights and fluorescent UVB tubes are present and have been positioned to allow suitable animal exposure. A hemp-bark substrate has been used and food is offered on tiles, which are easily wiped clean. A radiator heating the ward is clearly visible in the background and a solid-tiled floor gives the insulated room a high degree of thermal inertia.

Fig. 9.5 Vivarium furniture should be simple and unique to each animal. Here a *Testudo graeca graeca* is housed.

The vivarium illustrated has a fluorescent strip light, two basking spot lamps, a maximum-minimum thermometer, a humidity gauge, feeding tiles, a bathing tray, two plastic hides (orange—beneath the bathing tray), hemp substrate, a rock to provide some thermal inertia (to be discarded after use with this animal) and disposable gloves to reduce the risk of human and animal contagion.

Handles allow the vivarium to be carried and placed outside during good weather.

Fig. 9.6 Larger vivaria suited to species such as *Geochelone pardalis* are also ideal for accommodating snakes and larger lizards. These are also fitted with basking and ultraviolet lamps. Misting, damp towels and hide placement will help increase humidity where necessary.

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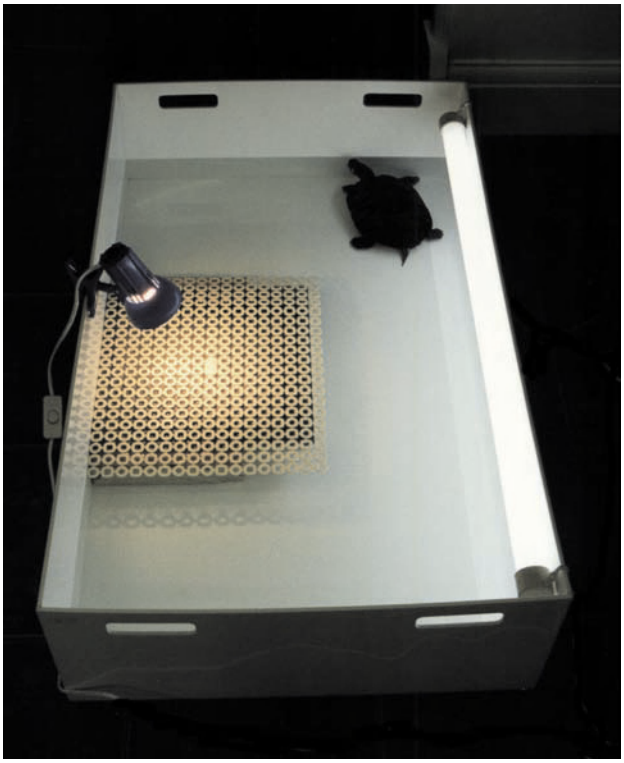

Fig. 9.7 A hospitalisation tub is easily converted to accommodate a semi-aquatic species such as this *Trachemys scripta elegans*. Here a basking area is constructed using bricks and a tile. Below it the animal is able to shelter within a hide.

Electrical circuits should have circuit breakers in order to reduce the possibility of operator or animal electrocution. A ramp will assist the animal haul out of the water and feeding can be undertaken in a separate tank or tub table.

Fig. 9.8 In order to avoid excessive heat trauma, smaller animals and animals recovering from anaesthesia require close monitoring if placed beneath basking lights.

- Substrate should be changed regularly.
- Food should be provided on tiles or in dishes to reduce the chance of ingestion of substrate with food items.
- Food should be provided away from direct basking areas, or before basking lights are switched on, as this will reduce dessication of food items.
- A hiding box should be provided for most mobile terrestrial species.

Fig. 9.9 Plastic plants require no maintenance and are easily disinfected between animals by soaking overnight in appropriate disinfectants. Keeping a varied supply of such plants ensures that animals can be given shelter and environmental enrichment during their recovery. Use of plants also allows a higher humidity to be provided when basking lights are in operation.

Fig. 9.10 Shelters, such as the orange plastic tunnel hide shown here, will protect mobile animals from excessive heat and light exposure.

Where small animals, which may instinctively fear bird predation, are housed, several hides should be placed about the vivarium. Such animals often appear distressed as they bury themselves or cling to the vivarium perimeter with their heads hidden when hides are not provided. Multiple hides encourage the animals to explore without fear as they can move from shelter to shelter.

- Multiple hides should be considered or provided for hatchlings and neonates, as these tend to be instinctively reclusive to protect against possible predation. Without suitable hides many of these animals display distress behaviour.
- Where chelonians are inactive and unable to remove themselves from basking heat sources, or where there is limited room within a vivarium set-up, intermittent hide placement may help to protect the animal from excessive exposure to heat sources.
- Often, severely debilitated chelonians are incapable of movement. In this state, a small environment with no furniture will be sufficient. As an animal recovers, the vivarium environment must take into account the changing needs of the patient. This must include behavioural requirements.
- It is best to hospitalise basking terrestrial chelonians from arid environments within open-top vivaria, generally heated from above using basking lamps and daily heat and light cycles.

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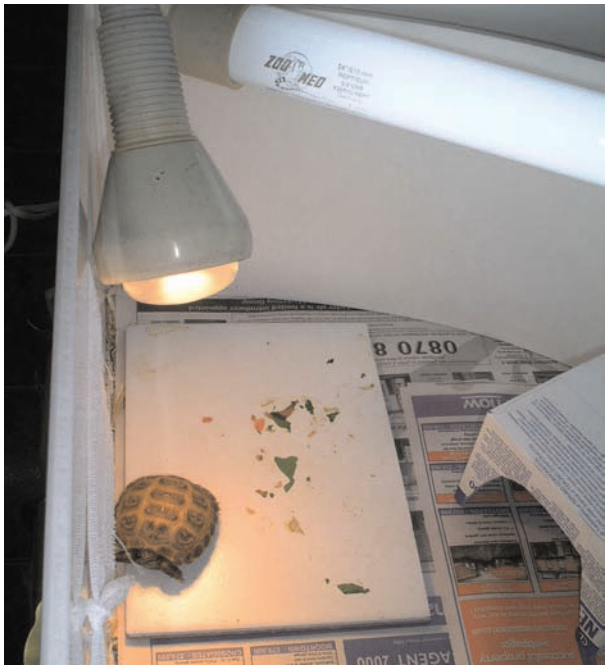

Fig. 9.11 Cutting entrance doors into small boxes produces simple disposable hides for small neonates.

Plastic containers, such as upturned margarine tubs with lids *in situ*, can be used to create small humidity chambers. Sponge can be glied beneath the plastic roof and this can be regularly moistened to create humidity without compromising hygiene.

Fig. 9.12 Where purpose-built vivaria are unavailable, glass tanks or other receptacles can be used as substitutes.

Here a five-litre water bottle is filled with hot water and then placed on a heat mat. This provides background heat, which is complemented by the two clip-on desk lights that provide basking heat. Care should be taken to ensure that ventral heat provision is not excessive. An ultraviolet tube is provided. Newspaper substrate is flammable and requires careful monitoring as it poses a serious fire threat.

- Species that have a high humidity requirement may be best hospitalised within closed-top tanks which are misted on a regular basis.
- Photophobic terrestrial species should not be forced to bask. Generally these are best nursed in shaded vivaria maintained possibly in conjunction with high humidity through misting and at a more constant temperature if the species requires this.
- A bathing area should be provided for semi-aquatic species and a drinking area for most terrestrial species. There may be no need for a drinking area when a fluid administration and bathing protocol provide daily requirements, especially if such animals are also immobile.

Fig. 9.13 A similar set-up to that in Fig. 9.12. Again a 5-litre water bottle is heated by a pad and complemented by a basking source (desk lamp). A humidity and temperature gauge is used and bedding/substrate have been omitted to avoid the risk of fire.

This animal has its own bathing tray and is bathed regularly as no water source is provided because of size limitations. Without careful observation, this animal could be exposed to excessive heat.

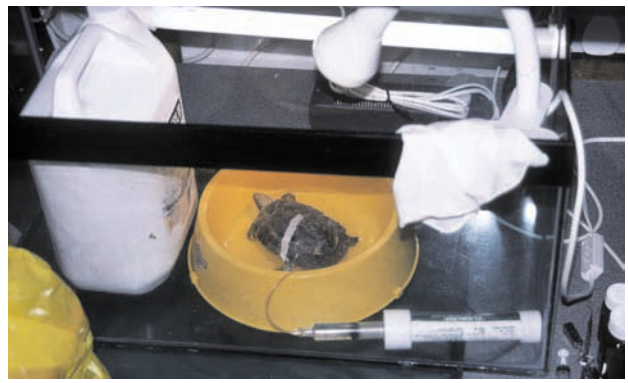

Fig. 9.14 Here an animal on an intraosseous drip is provided with a soak (bath). The basking lamp is not being utilised as that would unfavourably reduce humidity. Again, a hot water bottle and the bath are warmed by a suitable thermostatically controlled heat pad.

MANAGING THE VIVARIUM ENVIRONMENT

Heat

Enzymatic processes are temperature dependent. Important enzyme-controlled functions include most metabolic activities, including cellular delivery of energy, creation of body proteins and hormones, cell division and digestion of food. As a result of influences on peripheral stem-cell division and bone-marrow activity, even the effective functioning of the immune system is temperature dependent. Ambient temperature dictates the rate of all reptilian anabolic, digestive and homeostatic activities.

Most commonly encountered terrestrial herbivorous species utilise postprandial basking as an aid to digestion. A daily basking session under the sun or a tungsten bulb/light source will ensure that adequate core body temperature is maintained over time.

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Fig. 9.15 Glass tank vivaria can be used to accommodate species where regular misting and high humidity are required. A cover can be used to shield the tank and the use of lights avoided if the species is photophobic. Heat pads can be placed on side walls to create radiant background heat.

Fig. 9.16 This sealed vivarium has moistened peat as a substrate. Heat is provided by a low wattage indirectly placed basking lamp. A temperature gradient is created. Placing several hides within an enclosure like this will help maintain the animal without exposure distress.

The control and efficacy of the immune response to infectious agents will depend upon whether or not the animal is maintained within its ATR. For this reason we advise that all debilitated chelonians are maintained within their ATR. It is unwise to allow chelonians fighting an active infection, coping with metabolic disease or in the process of healing traumatic injuries to drop below their ATR as this may force metabolism to shift towards hibernation and impair immune and anabolic processes at a time when improvements are required.

With one possible exception (the leatherback turtle) chelonians are ectothermic reptiles. This means that they are dependent upon a combination of ambient environmental temperature and behaviour mechanisms, such as basking or burrowing, to regulate their core body temperature. It may seem an obvious comment, but supplementary heat is not necessarily required if chelonians are nursed in their area of natural origin. In that case, it may only be necessary to provide supplementary heating during cooler seasons, but not during hotter periods. In more temperate environments (non-equatorial northern and southern latitudes) supplementary heat is crucial where ambient temperatures are far below a reptile's preferred body temperature.

The type of heating system used for a reptile vivarium/aquarium will depend on the species preferences that can be identified. These are outlined in more detail in the earlier section dealing with environmental husbandry.

When hospitalising primarily *Testudo* spp., the temperature within the author's (SM) ward does not fall below 20°C at night. It is allowed to reach 32°C for moderate periods every day. This is generally during and after feeding which in its turn follows bathing. The ward has a solid tiled floor to stabilise temperature through thermal inertia and it is double-glazed. Within this ward the vivaria are heated in a variety of ways depending upon the

Fig. 9.17 Photophobic species, such as this juvenile hingeback tortoise (*kinixys erosa*) enjoyed periods of very high humidity. This is achieved by the use of moistened peat substrate, regular misting, shielding of the tank from external light and the absence of a basking lamp.

Food is often preferred in a blackened and almost rotting state, as opposed to the fresh state enjoyed by most humans.

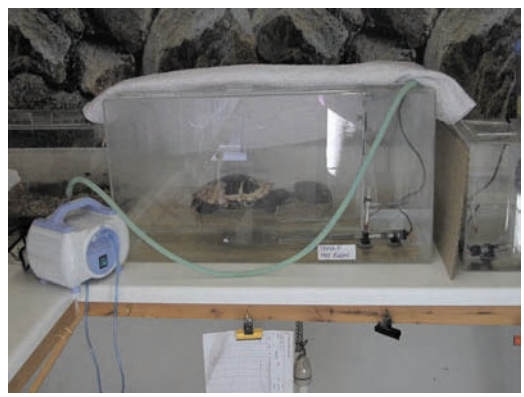

Fig. 9.18 Here an Aquabrooder® and nebuliser are used to deliver antibiotics to this semi-aquatic chelonian suffering from lower respiratory tract disease.
(Courtesy of Aidan Raftery)

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Fig. 9.19 Cat and dog cages without supplementary heat and light provision, soft bedding and a diet of cat food are all completely inappropriately offered to this herbivorous basking *Testudo*.

Fig. 9.20 Here an anaesthetic recovery tank has been set up within a dog cage in order to provide heat and light to a basking chelonian recovering from general anaesthesia. Heat pads, heat bottles and a basking lamp ensure that the animal maintains a suitable body temperature.

Fig. 9.21 A maternity vivarium is created by filling a tub with peat and covering it with a sheet. This animal has been induced and an egg has already been passed. It is stored in the polystyrene box on the front left hand side of the vivarium and will then be placed into the incubator to the right of the tub.

species. On the ward, the cloacal core body temperature of healthy *Testudo* and *Geochelone* spp. allowed to bask for 3–4 hours a day, measured using a digital thermometer probe attached to a pulse oximeter, is always above 26°C, both day and night.

Semi-aquatic chelonians may benefit from combinations of water heating and basking areas depending upon species. Marine species are generally hospitalised *in situ* in warm latitudes where supplementary water heating is not required. Cold-stunned animals and those washed up in more inclement environments will benefit from appropriate heat provision.

Fig. 9.22 An example of an incubator that is easily converted to take chelonian eggs. Many owners of hospitalised gravid chelonians will expect eggs to have been appropriately handled and incubated prior to collection, following induction of oviposition in a fertile female.

Fig. 9.23 A semi-aquatic chelonian is hospitalised beneath basking and ultraviolet lamps. Plastic slatted trays reduce exposure to water when skin and shell lesions are present. A ramp and a plastic hide are visible in the foreground. All vivarium furniture is easily disinfected between animals.

Monitoring vivarium temperature

Use maximum—minimum thermometers, or digital thermometers, within all hospital enclosures, at least while setting them up. Remember that increased background temperatures, such as a spell of sunshine and fair weather, may alter the conditions within the vivarium. Without some method of monitoring this, it will not be known what conditions the patient is actually being exposed to throughout its treatment. Further thermometers can be used to monitor the background temperature of the ward within which enclosures are placed.

Providing heat in a hospital environment

Where possible, heat should be provided using thermostatically-controlled heat sources. This is often easy in an enclosure of permanent construction, such as those provided by many quality

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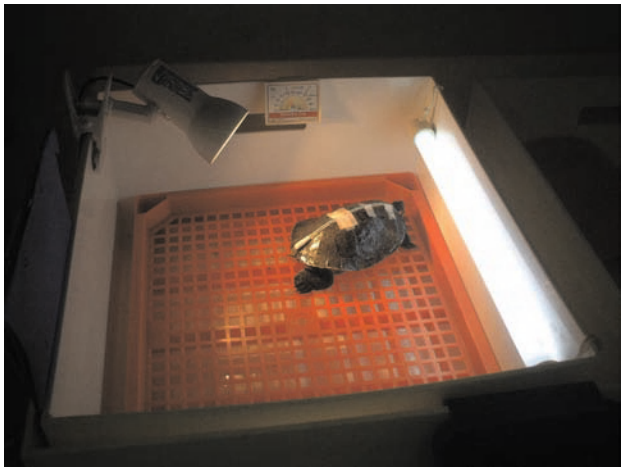

Fig. 9.24 Dry-docking a semi-aquatic turtle, a red-eared slider (*Trachemys scripta*).

This turtle is on slats above a water bath. The below-floor water source, in conjunction with regular misting, maintains humidity.

This sort of set-up is ideal for the stabilisation of bacterial infections of the shell (SCUD) and following coeliotomy or shell surgery.

Fig. 9.26 Large, aggressive species, such as this snapping turtle (*Chelydra serpentina*), can be temporarily housed in tub-table tanks.

It is important that a grill is placed over the top of the tanks and that it is adequately weighted to prevent escape. Signs should clearly inform all staff that an aggressive and dangerous animal is contained within the tub.

Fig. 9.25 Here a red-eared slider (*Trachemys scripta*) with an oesophagostomy tube *in situ* is allowed to swim and feed in a tub table tank.

Separating feeding and exercise tanks from normal hospitalisation tanks maintains hygiene levels.

Fig. 9.27 Animals can be given temporary outdoor exposure and grazing in meshed enclosures. However it is hard to guarantee that cross infection from ground contamination will not occur, and this author (SM) prefers to use plastic hospital tubs with handles (Fig. 9.5), as these can be carried outside to allow the animal to bask.

Fig. 9.28 This animal intensive care unit (AICU) is ideal for maintaining precise temperature and humidity in hospitalised reptiles.

The Electronic Animal Intensive Care Unit (AICU®)

Lyon Electrical Company, c/o HotSpot for Birds, 27 Essex Drive, Northridge, CA

(Courtesy of Stephen Hernandez-Divers, University of Georgia)

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keepers, but it may be unworkable in a veterinary environment unless the through-put of cases warrants it.

Heat sources are generally one of two types: primary sources that are used to regulate the background temperature of the vivarium; secondary sources used to alter the moment-to-moment temperature of the local vivarium environment.

In most terrestrial enclosures, as in natural environments, cycles of heat and light are intricately linked. Diurnal heat cycles appear potentially beneficial to most recovering chelonians. The duration of peak or full heat provision can (and generally should) be varied in relation to the photoperiod as in Table 5.3 (Jones 1978). This author (SM) advocates that 50% of the photoperiod be at or above the patient's PBT. Alternatively, heat periods can be provided as the clinician feels suitable to the individual patient.

Electrically-heated hot rocks and ventral heat pads are not particularly appropriate as the main heat source for most chelonians, which tolerate ventral heat sources poorly. Heating from below should be avoided in preference to all-round heat provision in non-basking species. Significant plastron, digestive tract and skin damage is regularly caused by excessive heat and contact with hot faeces or urine.

Background ward heating systems, heat pads and hot-water bottles are common methods of warming enclosures for terrestrial species. These act as the primary heat source described above. Temperatures from primary heat sources should not be allowed to fall excessively at night in order to maintain a treatment vivarium within the bottom end of the patient's ATR.

Basking lights, infrared ceramic heat bulbs and thermostatically-controlled surface heat mats (especially those which can be applied to side walls as opposed to floor surfaces) are suitable for achieving a daily thermal cycle in addition to background heating. These are the secondary heat sources described above. Sunshine is potentially the best source of radiant basking heat for reptiles (when available).

Substrates such as paving slabs and tile floors maintain their temperature after they have been suitably warmed and can be most useful when placed below basking lamps. These act as simple heat storage mechanisms, and they give out heat slowly after the basking lights are switched off. This thermal buffering of the vivarium is different from ventral heating with electrical heat sources mentioned above, as warming of stone within a vivarium actually reduces ventral heat loss from the patient, as opposed to cooking it!

Heat loss

Heat is lost through conduction, convection and radiation. Open-topped set-ups are prone to high rates of loss, whereas insulated enclosures with reduced ventilation lose heat slowly. Use of heat stores, such as solid flooring, is helpful, as is placing a cover over a vivarium at night.

Problems associated with inappropriate heat sources

Reptiles lack the ability to shield themselves from extreme heat and are therefore highly susceptible to thermal burns and hyperthermia. Immobile patients are particularly vulnerable. Hyperthermia commonly occurs when there is a weather change and unexpected exposure to the sun adds to background heating.

Dermal lesions will develop on an immobile patient with inadequate sanitation, especially where faeces are allowed to remain in contact with the animal. Directly heating patients from below should be avoided. Chelonians tend to sit on warm spots, allowing faecal and urinary material to incubate below them. This predisposes towards plastron infections especially where humidity is high, the more so where the patient is immobile. Many heat pads become unreliable with age and may contain excessive hot spots, or overheat, and must be used with great care if at all.

Aquatic environments should have heat sources shielded from aggressive animals or physical trauma. The risk of electrocution to staff and patients should be carefully evaluated. Power sources should be appropriately fused and cables should be able to resist beak bites.

Photoperiod and light

Bartholomew (1959) suggests that photoperiodicity is important in the normal physiology of most reptiles, with the possible exception being those living near the equator. It is likely that, in conjunction with temperature cycles and endogenous factors, photoperiod acts as an environmental stimulator of hibernation physiology and reproductive function. Species requirements should be researched. It should be remembered that some tropical species are photophobic. Here a basking set-up with additional ultraviolet exposure will seriously compromise the health and viability of the patient.

This author (SM) routinely exposes all basking reptiles to reptile-specific UVB-emitting fluorescent tubes or other light sources. These suit most basking reptiles and complement infrared and tungsten-bulb heat sources. Expose to direct sunshine is also provided whenever possible. Remember that glass and plastic filter out useful UVB and there should be nothing between the reptile and the sun unless it is guaranteed to allow transmission of useful UVB wavelengths.

- UVB emitting fluorescent tubes must be replaced according to manufacturer's instructions.
- Photoperiod charts should be followed.
- Hides and darkened vivaria are provided to species considered photophobic (here heat is usually provided by a method other than basking).

Jarchow (1988) points out that constant exposure to light is stressful and advises that the natural light preference of captive reptiles should always be considered. Captive reptiles should be offered shelter from excessive exposure to light. This is essential where they are unable to move of their own accord. Regular patient observation is essential.

A chaotic photoperiod or sudden alteration may affect reproductive and other processes. An example of a photoperiod chart suited to *Testudo* spp. (Jones 1978) is given earlier in the captive environment section (Table 5.3). This author's clinic (SM) follows a cyclical photoperiod by resetting timer devices on lighting circuits every 2 weeks.

Decreasing photoperiod may induce hibernation preparation and decrease anabolic activity. Therefore, when hospitalising debilitated chelonians, extending the photoperiod and the associated heat period can be beneficial in spring and autumn/fall when anabolic activity would be low or decreasing.

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Humidity

There are vast differences in humidity tolerances of different species. A general guide is given in Table 5.1. Chelonians may come from arid terrestrial, humid terrestrial, semi-aquatic and aquatic environments. It is therefore essential to consult up-to-date sources of information giving details on the appropriate care and environmental preferences of more exotic species. Inappropriate humidity will at best impair recovery from disease, at best will result in behaviour alterations, affect skin shedding and quality and predispose to dehydration, anorexia and death. Humidity-loving species, such as many Asian turtles, often enter terminal decline when placed into a dry, brightly-illuminated vivarium with basking lamps. Arid-loving species develop dermatological lesions, respiratory and other infections and decline if kept too damp.

It is possible to establish and measure a humidity gradient across a vivarium. Where the preferences of a novel species are unknown it is reasonable to experiment with the environment that is provided. We have offered mixed environments to chelonians and achieved dramatic improvements in health through simple alterations in humidity. In the United Kingdom, this author (SM) has encountered Asian chelonians of different species purchased simultaneously from market stalls requiring opposite extremes of humidity.

Provision of humidity

A combination of misting, water sources and damp substrate, such as paper towels, bark mulch or sphagnum moss, will provide humidity. Removing vivarium furniture, increasing ventilation by removing covers and circulating air and the use of basking and other heat sources will reduce humidity.

Monitoring humidity

Humidity gauges are relatively cheap and can be obtained from most garden centres. Our clinic uses both simple plastic devices and digital monitors. These are easily placed within each vivarium. With experience of species needs and the vivarium set-up humidity gauges may become less necessary.

Species from arid terrestrial environments enjoy a low humidity of 40% or less. Short periods of bathing are provided each day, followed by feeding and basking at low humidity.

Tropical species often enjoy a high humidity, greater than 60%. By closing down ventilation, maintaining adequate background heat and regular misting a humidity of 90%–100% is easily obtained.

Furnishing

Captive reptiles should be provided with cage appliances that simulate important components of their natural microenvironment. These should encourage normal behaviour and reduce stress associated with captivity. In the hospital environment these need not be as elaborate as in a long-term terrarium (Jarchow 1988). We try to avoid putting anything into a vivarium if it doesn't need to be there for the well-being of the in-patient.

Some points to bear in mind when furnishing a hospital vivarium:

- all hospital furniture should be disinfectable and/or disposable;
- substrate may need careful selection to avoid problems associated with fire risk, ingestion, wound management or clinical waste management;
- a hiding box should be provided for most mobile terrestrial species. Placement of shades and shelters may be intermittent depending on heat sources that are employed;
- a bathing area should be provided for semi-aquatic species and a drinking area for terrestrial species.

HOSPITALISATION VIVARIA

TERRESTRIAL CHELONIANS

Historically this author (SM) has seen terrestrial chelonians maintained successfully in a wide variety of temporary enclosures. These have included: a fish tank; a self-build kitchen-cupboard unit, with doors and shelf removed, placed on its back; a plastic storage box; a strong cardboard box; a gardeners' propagator and a purpose-built vivarium. Ideally, vivaria should be constructed out of non-porous, non-abrasive, disinfectable materials, as previously described. Ventilation and disinfection are primary construction considerations.

During hospitalisation, most species benefit from maintenance within a range of 20°C–30°C, but ATR/PBT and thermoperiod preferences of individual species should be researched, and conditions tailored to meet individual needs. We advise the use of a simple, maximum–minimum or digital thermometer within the vivarium. This can help prevent conditions that could induce thermal stress. Some species-specific data on appropriate vivarium temperature ranges are available in Table 5.1.

Where weather permits, animals should be placed outdoors and allowed to bask. This achieves remarkable psychological and physiological benefit, with increased activity and appetite. It may also assist with calcium metabolism and other metabolic processes. Outdoor enclosures must be constructed to prevent cross-infection of patients or become single-use only. Transportable indoor-outdoor vivaria can be moved outside without the need for repeated disinfection.

Low-humidity-loving basking species (most *Testudo* and *Geochelone* spp.)

Heat

It is general practice to hospitalise terrestrial temperate/arid environment chelonians in open-top vivaria, heated from above with basking lamps. Basking species enjoy radiant heat and light, especially after meals during periods of digestive fermentation. Basking heat sources such as infrared bulbs, ceramic heat bulbs or high wattage tungsten bulbs are all appropriate. Combined ultraviolet/basking heat sources are becoming more available (e.g. PowerSum® 200-med) and this author has met with good results in chelonians such as *Geochelone pardalis* and *Testudo* spp.

The use of a solid floor on a reptile ward is desirable. These have a high degree of thermal inertia so a stable background temperature is created. Within vivaria, single-use rocks, concrete

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and paving slabs, placed below a radiant heat source will heat up during the day but cool slowly at night when the radiant source is disconnected. This will buffer heat loss. However, paving slabs and rocks are awkward to disinfect, especially where there is a high through-put of cases through the ward. Here it may be best to discard them, or to control background temperatures with other methods.

Whilst enclosures are best ventilated by having an open-top (box) structure, a lid or covering at night will slow heat loss and prevent excessively rapid cooling. Shaded bulbs, ceramic bulbs, and red/blue coloured tungsten bulbs can be used at night to provide background heat with minimal illumination. Where these are used it is normal to provide visible and ultraviolet-spectrum light during the day to create an appropriate day and night illumination difference.

Heat pads can be used cautiously to maintain night temperatures within enclosures, bearing in mind their potential disadvantages when used with chelonians. Heat pads are best placed on vivarium side walls, to achieve a source of radiant heat.

Substrate

Substrate within the vivarium should be non-irritant and should not cause impaction if ingested. Our clinic favours the use of compressed, baled, hemp chippings. This is manufactured as bedding material for horses with chronic airway disease. Hemp is highly absorbent and will receive large amounts of urine and faeces without becoming unmanageable. This allows nurses to remove soiled areas on a daily basis and completely refresh enclosures once or twice weekly as necessary. This substrate is also unlikely to ignite under basking lamps, is well tolerated by chelonians and produces only moderate amounts of dust if changed regularly.

Full-spectrum lighting (FSL)

Outdoor enclosures should also be provided in sunny areas wherever possible. The use of transportable indoor-outdoor vivaria, as used at our clinic, means that electrical heating systems can be removed and the vivarium carried outside without excessive need for endless and repeated disinfection.

We advocate the use of full-spectrum lighting (FSL) (e.g. Reptisun® 5.0 or Powersun®, Zoomed) in all our low-humidity vivaria. Some juveniles exhibit photophobia when first exposed to FSL and these animals benefit from hides or reduced FSL photoperiod. Photoperiod is discussed more fully in the earlier section on husbandry.

Water

Water should be provided, either as a drinking area or through administration of fluids and a daily bathing regime. Fluid administration and daily bathing may reduce the need for a source of water in severely-debilitated specimens, especially if they are immobile. Frequency of urine output can be used as a guide to adequate access to fluids. Most terrestrial chelonians at our clinic will urinate at least once every three days during periods of prolonged confinement. Those eating succulent foods and in reasonably good health would be expected to urinate daily or every second day. We routinely administer 0.5%–1% of body weight (in millilitres) of fluid to any hospitalised chelonian to guarantee appropriate renal function.

High-humidity-loving non-basking species (e.g. African hingeback tortoises)

Heat

These animals do not enjoy radiant basking heat sources and prefer a more even background heat. Hides and humidity chambers should be offered (an upturned plastic ice cream or margarine tub with an opening cut into it would provide perfectly adequate cover). Heat pads and high ambient background temperatures along with heated water in bathing areas are more appropriate, provided the animal is protected from heat trauma.

Where heat tubes are placed in water, they should be shielded to avoid damage from strong and potentially aggressive specimens. Fused circuit breakers are crucial to eliminate the possibility of staff or patient electrocution. Background temperatures may have less diurnal variation than with basking species and at this author's clinic (SM) are commonly 22°C–28°C.

Humidity

Misting and dampening of substrate will help increase humidity. Ventilation tends to be of lesser importance, and humidity is easier to maintain if a lid or door can close the vivarium for a significant proportion of the day.

Water

Water should always be provided, either as a bathing and drinking area, or through administration through gavage or oesophagostomy tube where this is unrealistic. Where evaporative water losses are likely to be high, misting and fogging should be undertaken on a regular basis as suggested above.

Full-spectrum lighting

Many tropical ground-dwelling species are moderately photophobic. Few of these animals benefit from FSL. Where FSL is used, appropriate shelter and photoperiod should be provided.

Substrate

Choice of substrate will depend upon the behaviour patterns of the species, the likelihood of lesions becoming contaminated with substrate and the ability of the patient to move around. More mobile animals will enjoy a dampened soil or peat/sand base. This should be free of pesticides and fertilising chemicals and attended to regularly to reduce sporulating fungal colonisation. Animals should be carefully observed for substrate ingestion and where this occurs substrates able to produce intestinal impaction should be removed.

Immobile animals may be housed within a simple tank or other vivarium on a dampened towel, which can be washed or discarded between uses. Plastic draining board mats may help limit ground contact in humid environments where soil and excreta can predispose to dermal and plastral infections. This is especially true of patients with limited movement and open wounds.

SEMI-AQUATIC CHELONIANS

Small species (<5 kg)

Debilitated, semi-aquatic chelonians can be kept out of water in a humid environment until the risk of drowning has passed

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(Figs 9.7 & 9.23–9.25). When they are kept out of water, regular misting is advisable.

Plastic draining board mats may help limit ground contact in humid environments where excreta can predispose to dermal and plastral infections. This is especially true for patients with limited movement.

Many species will benefit from basking heat sources placed over any haul-out area, but patients must be carefully monitored with regard to potential thermal injury if they are unable to escape from basking sources. Lighting circuits must be isolated from water.

Larger species

These animals may be capable of inflicting serious injury upon staff and may escape from confinement where containing vessels are of inadequate strength. Bathtubs and veterinary tub tables are a great help. Tub tables allow the convenient use of bath taps and shower units to provide adequate water and humidity (Fig. 9.26). Glass containers must be avoided. Lids or mesh should be kept in place and should be appropriately weighted in order to prevent escape.

Where the animal has significant exposed injuries, reduced exposure to water may be necessary and tiled pits with appropriate plug fixtures and shower units employed. Plastic wading pools for children also work well.

Heat tubes that can be damaged or smashed by the patient and electrical cabling should be avoided. High background heat, warm-water sources and basking lamps where appropriate, can all be used to supply heat. Sealed five- and ten-litre plastic containers can be filled with hot water and immersed in tub table water to maintain water temperature overnight.

Warning signs explaining the danger of injury should be clearly displayed so that staff members do not encounter the animal without prior warning. Protective gloves are advisable.

MARINE CHELONIANS

Generally, facilities for long-term hospitalisation are either net-based flotation tanks or large, plastic, land-based tanks (Figs 9.29–9.37) Shower units and bathrooms can be utilised for short-term emergency stabilisation.

Fig. 9.29 Marine turtles can be nursed in purpose-built fibreglass tanks. Continuous water flow and an external sand filtration system ensure water quality is maintained.

Fig. 9.30 A turtle is hospitalised in an isolation tank during initial assessment and stabilisation. Following admission for extensive external fibropapillomas, the discovery of multiple visceral lesions through diagnostic imaging techniques may necessitate euthanasia.

Fig. 9.31 Isolation tanks suitable for housing marine chelonians with infectious diseases must have an individual water supply and tank water must be constantly filtered in order to reduce re-exposure to pathogens.

Fig. 9.32 Waste water should be regarded as infectious to other turtles and efforts should be made, through filtration and disinfection, to avoid contamination of other enclosures with waste water.

Some general points about handling marine turtles:

- Flippers should not be used as holds to pick up turtles. The body of the turtle should be used wherever possible. It may be necessary to use slings and winches for large specimens.
- Turtles out of water should have their plastrons rested on foam where possible, as they will no longer be supported by water. A turtle should not be placed on its carapace.

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Fig. 9.33 After stabilisation and treatment for traumatic conditions, rehabilitated turtles can be maintained in a landscaped communal exercise/swimming pool for a period of as long as a year before assessment for future release.

Fig. 9.34 Flotation tanks offer a simple alternative to the complex hospitalisation facilities above. Facilities designed for farming fish are easily converted.

Fig. 9.35 Water quality and temperature variations of local water current are important and must be considered when locating flotation tanks. Inappropriate temperatures and poor water quality will predispose to disease and reduce recovery rates.

- Transportation to and from treatment stations should be carefully planned so as to prevent unnecessary exposure to heat and desiccation. Vehicles are best shaded as opposed to open topped.

Fig. 9.36 Hospitalised marine turtles adjust to institutionalised feeding regimes. Dietary requirements require detailed consideration.

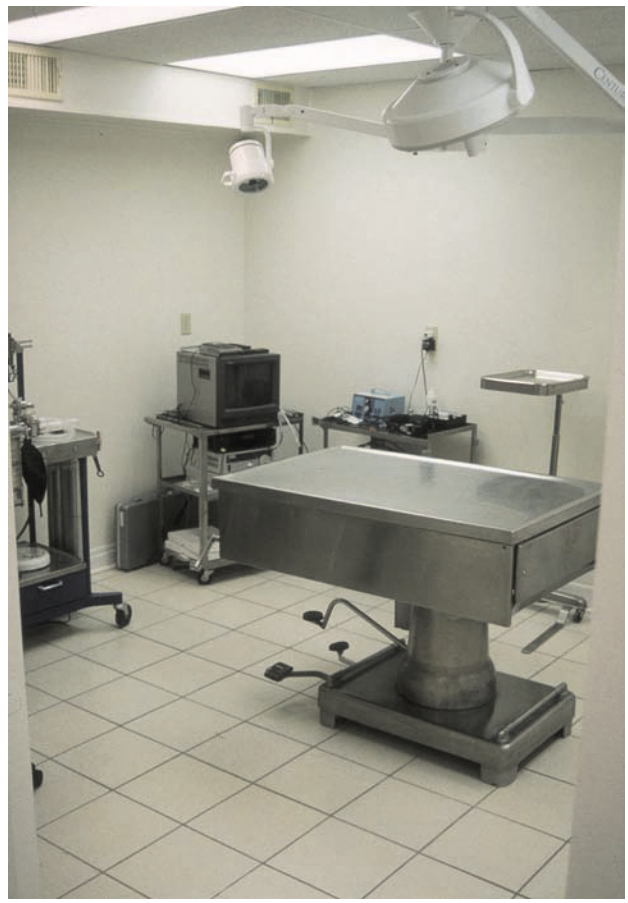

Fig. 9.37 The examination facilities at Marathon Turtle Hospital, Florida, are extensive and include equipment for anaesthesia, radiography, endoscopy and blood assessment.

- Inactive, debilitated, traumatised animals, along with turtles recovering from general anaesthesia, should be kept out of water until the risk of drowning has passed.

Land-based tanks and pools

Filtration systems are generally required to maintain water quality and to reduce bacterial contamination. Care should be taken

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to ensure that water containing infectious agents is not knowingly discharged directly into the sea.

Active animals may cause damage to tanks and pools if confinement is inappropriate and animals are bored. This can lead to ingestion of foreign material, impactions and intestinal disturbances. Animals should be permitted to swim freely, and have access to exercise pools wherever possible during their recovery.

When constructing a hospitalisation tank, avoid including corners or ledges where reclusive debilitated animals may withdraw and entrap themselves, as this may cause drowning.

Where turtles are hospitalised at a latitude of origin, supplementary heating is not generally required. Small tanks, however, may lose heat overnight if inadequately insulated. Tanks should also be shaded from direct exposure to the sun, as animals will be unable to escape its radiant heat and may become heat traumatised.

Flotation tanks

Flotation tanks are best placed where there is a continuous flow of water. This will reduce bacterial contamination caused by excessive build up of faecal material below the tanks. Alternatively tanks can be relocated on a regular basis. Care should be taken when nursing animals harbouring infectious agents as these may become concentrated and are often discharged directly into the sea.

Apart from the above, care in flotation tanks is as for land-based tanks and pools.

HOSPITAL CARE

HOSPITALISATION CARE PLANS AND IN-PATIENT FORMS

In a ward environment, all hospitalised chelonians should have a standardised and comprehensive care plan. Tick boxes and comment sections ensure that nursing is appropriate during periods of hospitalisation.

The nurses at this author's clinic (SM) are happiest with reptile clinicians when care plans detailing short-term, medium-term and long-term diagnostic and therapeutic aims are made and updated on a regular basis. With the aid of such care plans it is easier to nurse and assess the progress of cases.

On admission, the short-term aim is generally to undertake appropriate investigations to achieve a diagnosis. The medium-term aim is generally to turn the proposed condition around through stabilisation and corrective therapy. The long-term aim is to foster recovery and correct errors in husbandry and care that may have predisposed to illness in the first place. Such aims and proposed timescales to achieve them must be frequently updated in response to patient condition, ethical considerations and the keeper's wishes.

Keepers can be provided with a comprehensive, individual care plan when the patient is discharged. This ensures the health of their tortoise continues to improve in line with expectations.

It is essential that novice staff have the opportunity to observe normal and ailing reptiles in a variety of environments in order to become experienced in the management of sick reptiles. Hospitalisation allows appropriate observation over time.

Table 9.1 Recommended observation parameters.

- Weigh at least every other day
- Alertness, appetite, ability to prehend and swallow food, mobility, lameness and gait, ability to avoid obstacles (this may be suggestive of impaired visual capability)
- Visual reflexes (e.g. ability to track objects/menace response, pupillary reflex)
- Abnormal respiratory movements and rate (suggestive of respiratory disease)—open-mouthed breathing is abnormal (unless it occurs as part of a threat response)
- Abnormal vocalisation or respiratory noises (suggestive of respiratory disease or pain)
- Excessive neck extension (suggestive of respiratory disease)
- Neurological abnormalities or signs (such as circling/abnormal reflexes)
- Abnormal secretions and their rate of production
- Abnormal flotation in semi-aquatic/marine species (suggestive of respiratory disease, intestinal obstruction or other intestinal disease, solid coelomic lesions or gas/air within the coelomic cavity)
- Faeces (texture, frequency etc.)
- Urine (texture, frequency etc.)
- Cloacal tenesmus (suggestive of oviposition, cloacal organ prolapse, cloacal or cystic uroliths, cloacitis, etc.)

A hospitalisation form should be used to record observations. A minimum would include appetite, activity, urination and defecation. Table 9.1 lists the parameters this author (SM) recommends be observed and recorded.

In the eyes of many keepers of pet chelonians, small captive chelonians exhibit individual behaviour traits. They are often considered to have both character and personality by their keepers. Such traits can only be understood through contact with the animal over a period of time and this will require continuous observation at home or in an appropriate environment. This concept is also true for both nursing and veterinary staff that may need time to meet and understand the reptile they are treating. Often this understanding is of great importance to the keeper.

STAFF

A reptile ward is labour intensive and standards are dependent upon the skills and experience of its staff. Reptile care is very different from the routine management of cat and dog patients and staff highly skilled in one area of veterinary practice may unfortunately be lacking in other skills. Appropriate training and observation of staff in a reptile ward is essential. Once a substantial number of reptiles are being cared for it becomes easier to establish protocols and train staff. Staff time becomes far more effective. Many things become easier with the 'economies of scale' e.g. food preparation, assisted feeding, heating and nursing routines.

It is unwise to delegate responsibilities to staff with limited understanding of the animals in their care. It is important to explain to them that this is a skill that must be learned and to encourage them to become more experienced.

It is likely that a dedicated member of staff will be required to manage and monitor the ward. The expenses involved in this should be recovered through hospitalisation charges. Where keepers are unwilling to pay for hospitalisation, the facilities that

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can be provided to them will be limited. Pet insurance may well be the way forward in this regard.

LIMITING THE RISK OF INFECTION

The vivaria required for nursing sick chelonians will differ from those suited to long-term captive management of healthy individuals. One of the main reasons for this is the need to disinfect treatment enclosures and isolate potential infectious agents. Infectious agents can spread from animal to animal or animal to staff or keeper.

A sick chelonian is highly likely to shed any carried agents. Whilst agents may be of limited pathogenicity in one species, transfer in a hospital situation to a naïve species from a different area of origin may result in serious disease in the new species. Control of cross infection and barrier nursing sick chelonians are of major importance. Without the facility to barrier nurse, this author (SM) would advise hospitalisation of animals from multiple sources be declined.

Barrier nursing

'A hospital enclosure should be free of hazards that may be sources of infection' (Jarchow 1988).

Chelonians are subject to a remarkably large number of infectious diseases, and there is strong evidence of disease carriage, disease latency, recrudescence and altered pathogenicity when species barriers are crossed. All tortoises should be barrier nursed to isolate potential pathogens (Fig. 6.1).

Assume that all tortoises carry disease even if they look well and that they can give these carried diseases to other naïve tortoises (and to you!). All tortoises should undergo faecal-parasite screening upon entry to the ward, and as and when commercial herpesvirus molecular screening tests become available I advise routine screening of all in-patients. If possible, animals should also be screened for further infectious agents upon admission. Examples of these include helminths, enteric protozoans, haematogenous parasites, mycoplasmas, viruses, external parasites, *Salmonella*, *Cryptosporidia*, etc.

The protocol described in Table 9.2 should be followed.

Table 9.2 Barrier nursing protocol.

- Visitors to the ward must not handle animals or their enclosures.
- It should be assumed that all hospitalised chelonians carry faecal zoonotic organisms such as *Salmonella* spp. This will automatically increase hygiene precautions when compared with the nursing of average mammalian patients as it is essential that staff do not contract disease from poorly-managed patients.
- Disposable gloves should always be worn.
- Disinfectable or disposable aprons and boots are occasionally required.
- All treatment tanks/enclosures/vivaria should be isolated.
- All treatment tanks/enclosures/vivaria should be disinfectable or disposable.
- All tortoises should be provided with their own vivarium furniture and bathing trays.
- All worktops should be constructed to allow disinfection after use.
- Footbaths may be required at the entry/exit point of an isolation ward.
- The container used to transport an animal should be wrapped in a bag and stored until needed.
- Outdoor enclosures and water from tanks used for semi-aquatic species should not be drained into areas occupied by other chelonians.

Disinfection and cleaning

The choice of routine disinfectant depends upon the agents to be eliminated, toxicity to chelonians and toxicity to staff and keepers. Animals and surfaces can be disinfected using simple surgical disinfectants. Jarchow (1988) suggests that chlorhexidine disinfectants are often ineffective against *Pseudomonas* and advises that iodine and hypochlorite-based products be used instead. At our clinic, we routinely use Trigene® (Healthcare 2000, UK). It has excellent antibacterial, antimycotic and antiviral activity without being toxic to operator or chelonian. All disinfectants should be rinsed off in order to prevent potential skin burns or irritation to the patient.

Some additional points:

- frequency of washing and disinfection is determined by need;
- substrate should be changed regularly;
- enclosures with high humidity need more frequent cleaning and disinfection;
- faeces and urine should be removed as quickly as possible;
- food should be removed to reduce rotting, however the need for some animals to feed overnight and in privacy should be assessed and provision should be made if necessary.

Water from semi-aquatic, aquatic and marine facilities

Where sick chelonians are maintained near areas occupied by free-ranging chelonians it is essential that any potentially infective material, such as stale water from marine turtle enclosures is not allowed to contaminate areas occupied by wild specimens. Appropriate processing will depend upon local resources.

MATERNITY FACILITY

It may be necessary to set up a specific vivarium away from the main ward for oviposition (Fig. 9.21). If the climate is suitable, an outdoor enclosure may be provided for the purpose.

RECOVERY PERIOD

An average chelonian case presented at my hospital will often require admission and stabilisation for 5–10 days, during which time a variety of diagnostic and therapeutic procedures will be performed. Further recovery will depend upon the reason for presentation and is often directly related to the amount of time the animal has been ill.

Where animals hibernate, the season in which the animal is presented may have important implications for its further management. Animals presented with chronic disease are often best over-wintered and hibernation prevented. Animals already committed to hibernating will require careful assessment in order to create a sensible care plan.

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As a rule of thumb, I advise that most keepers should expect a recovery period 4–6 times longer than a comparable cat or dog case. Many chelonians can only be stabilised and recovery is not realistic. In situations where a successful outcome to the case is not possible, or unacceptable suffering is anticipated, euthanasia may be necessary.

DISCHARGING THE PATIENT

All clients should be given a patient-specific care plan which tells them about feeding, temperature regulation, fluid management, record keeping, nursing of lesions and administration of medications. The care sheet informs the client when to contact the surgery to give a progress report and when to return with their tortoise for a follow-up appointment. The client is thus provided with a written guide to all the information necessary to achieve the maximum recovery possible.

Discharge forms will also protect the surgery against possible future litigation should that become necessary. It is always wise to keep a copy of all information given to clients.

At the point of discharge, go through the post-hospitalisation care protocol with clients. This will often involve giving advice or instruction on:

- bathing;
- feeding/force feeding;
- passage of stomach tube or oesophagostomy tube maintenance;
- lesion management;
- administration of appropriate medications including injections.

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FEEDING TECHNIQUES AND FLUIDS

STUART MCARTHUR

FEEDING TECHNIQUES

The feeding requirements of hospitalised chelonians differ with species, season and health status.

If the mouth works then use both it and the natural food for the species. However, it is common for a sick chelonian temporarily to have lost the ability to feed itself. In such circumstances specialised feeding techniques, fluid administration techniques, diets and fluids will be required.

Complete recovery diets, live foods, mice, manufactured foods for omnivores and in-house blended vegetable and dandelion puree should be available if needed. For nutritional support this author (SM) regularly uses liquid recovery diets for herbivorous and omnivorous chelonians (Critical Care Formula®, Vetark UK). Omnivorous and carnivorous chelonians tolerate dilute mammalian recovery diets (e.g. Hills A/D®, Colgate Palmolive), but these do not suit herbivores. A liquidised herbivore diet can be prepared by blending, liquidising or crushing plants with a commercial juicer. This may be stored, chilled in a refrigerator, for short periods only, as such food will tend to ferment.

Table 10.1 summarises feeding and fluid therapy techniques for terrestrial chelonians.

Where animals require hand and syringe feeding, stomach tube passage or feeding through a temporary oesophagostomy tube, the nurses should be provided with a care plan detailing how to provide whatever is necessary.

OESOPHAGOSTOMY TUBE

Oesophagostomy tubes (O-tubes) are ideally suited to chelonian patients, especially where repeated handling during treatment would distress the patient or would be hindered by the animal's strength and its ability to withdraw into its shell. Placement of an oesophagostomy tube dramatically improves our ability to nurse and manage the recovery of chelonians suffering from major or chronic disease.

Oesophagostomy tube placement is generally a quick and simple procedure and a variety of plates are provided to illustrate this. Once in place, an oesophagostomy tube allows parenteral medications, fluids and food to be administered with ease, therefore nursing time required in patients with O-tubes is generally short. As simple nursing protocols can be created for animals with oesophagostomy tubes, this may allow some keepers to nurse and treat animals in their home environments. Mass insertions

Table 10.1 Enteral fluid therapy/nutritional support for terrestrial chelonians.

Hand feeding (Figs 10.1–10.3)	• Food is placed in the tortoise's mouth, e.g. using small, blunt-ended forceps. • Medications or nutritional supplements can be given to relatively healthy animals, e.g. by wrapping leaves around them. • Some animals may become stressed by handling for feeding, and an oesophagostomy tube would be better in these cases. • Smell is an important appetite stimulant for chelonians, especially when their sight is impaired. Such animals can be encouraged to eat by crushing fragrant leaves and fruits between your fingers to release juices and smells and then to place the crushed food beneath the animals nose. Smearing juices on the rhamphotheca is also helpful. A typical sight-impaired *Testudo* patient quickly learns where food is and when it is provided using this technique and they can therefore become conditioned to feed in a daily routine.
Syringe support (Fig. 10.4)	• Liquid (water, medications or liquidised food) is syringed into the animal's mouth. • Small amounts may be given frequently. • This may involve significant animal handling on a regular basis; some animals may become stressed by this and would be better suited by oesophagostomy tube placement.
Stomach tube (gavage Figs 10.5–10.8)	• Excellent for short-term nursing but needs to be compared to the benefits of a temporary oesophagostomy tube in severely debilitated long-term cases. • An important method of supporting a sick chelonian, providing it is tolerant of handling and its head is easily captured.
Oesophagostomy tube (Figs 10.9–10.20)	• An oesophagostomy tube is this author's method of choice for stabilising patients with upper digestive tract disease, handling stress or long-term debility requiring repeated enteric medication, nutrition or fluids. • These tubes are generally well tolerated. • Most animals are unaware of their tube and will happily eat around it (Figs 10.21–10.22).

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Fig. 10.1 Instrument feeding.
A debilitated spur-thighed tortoise (*Testudo graeca*) is placed upon an upturned litter tray to simplify instrument feeding.

Fig. 10.2 Smell is important in stimulating chelonian appetite, especially where vision is poor, such as in post-hibernation frost damage. Many herbivores are stimulated to eat if food is crushed and placed before them allowing fragrances to be released.

Fig. 10.3 Dandelions provide excellent nutrition for most herbivorous reptiles. They can be picked and stored refrigerated before being offered to in-patients.

Fig. 10.4 Here a sight-impaired male spur-thighed tortoise (*Testudo graeca*) is syringe fed during its recovery from frost damage due to an inappropriately managed hibernation. Liquidised food can be offered to the animal, which can learn to accept such feeding. Here, smell may be an important factor in stimulating appetite.

Early in the management of such animals it may be necessary to open the mouth forcibly and to syringe the food in gently until the animal adapts and cooperates with the feeding process.

Fig. 10.5 Passage of a gavage/stomach tube: Mark a tube to half the plastron length. Lubricate it. Before passing a stomach tube always ensure that it is filled with liquid and not air.

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Fig. 10.6 Open the mouth. It can be kept open either by the use of a gag or by the placement of a finger or thumb.

The trachea is avoided by running the tube along the roof of the mouth and pharynx. A tube should slip in gently and force should be avoided. It is unlikely that the trachea will be entered as the opening is easily seen in the caudal aspect of the tongue.

Fig. 10.7 A 2, 5, 10 or 20 ml syringe can be attached to the Luer tip of a tube, and the syringe contents administered slowly.

Before passing a stomach tube always ensure that it is already filled with an appropriate liquid, otherwise air will be expelled into the digestive tract when the syringe is attached.

It is unwise to administer fluids quickly in case excessive dilation of the digestive tract occurs.

Fig. 10.8 Flush the tube through after use, with approximately double its volume of water (often about 2 ml).

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Fig. 10.9 This author (SM) has a dedicated oesophagostomy tube kit containing the instruments and materials commonly employed in oesophagostomy tube placement.

Fig. 10.11 Cut down over the protruding tips of the forceps using a scalpel, and push the tips through the oesophageal wall and skin. Local analgesia should be considered for the area of skin where an incision is made.

In the unlikely event of hitting one of the major vessels in this area, apply temporary pressure with a swab or plug of cotton wool. Alternatively tie off the vessel with a suture.

Fig. 10.10 Insert long-handled crocodile forceps through the mouth into the oesophagus, with the neck held in extension. Push them laterally to protrude out in the mid oesophageal region.

Important structures in this area include the carotid artery, the jugular veins and the cervical lymphatics. In many species these structures can be seen and avoided.

Fig. 10.12 Pick up the end of the tube using the forceps and draw it in through the skin, up the oesophagus and out of the mouth by withdrawing the forceps.

Fig. 10.13 Reverse the grip on the tube and pass it back down the oesophagus.

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Fig. 10.14 Continue to pass the tube until it is in the region of the stomach. This is easy to feel if you are gentle (pre-measuring the tube is helpful).

Fig. 10.15 Put a finger suture around the tube and anchor it to the skin of the neck. Alternatively, apply a tape-butterfly and then place mattress sutures through the tape and the skin of neck to anchor the tube.

Fig. 10.16 Take up the free end of the tube and lay it over the carapace. Apply a small strip of tape to the tube and secure this beneath the nuchal scute of the carapace.

Adequate nuchal scute anchorage generally prevents self-removal. Carry on taping with more strips as necessary over the carapace.

Fig. 10.17 Animals that repeatedly remove the tube can have it anchored in a watch spring fashion to the skin of the dorsal neck and nuchal area in order to restrict leg access. The author finds this more suitable than taping front legs back, as suggested in some texts.

Fig. 10.18 Front view of watch-spring looping of the tube and anchorage above and below the nuchal scute in order to secure the tube and reduce self-removal.

Fig. 10.19 Here protective tape has been used to reduce the chance of removal by the patient. The tube is well tolerated and relatively unaffected by flexion or extension of the neck.

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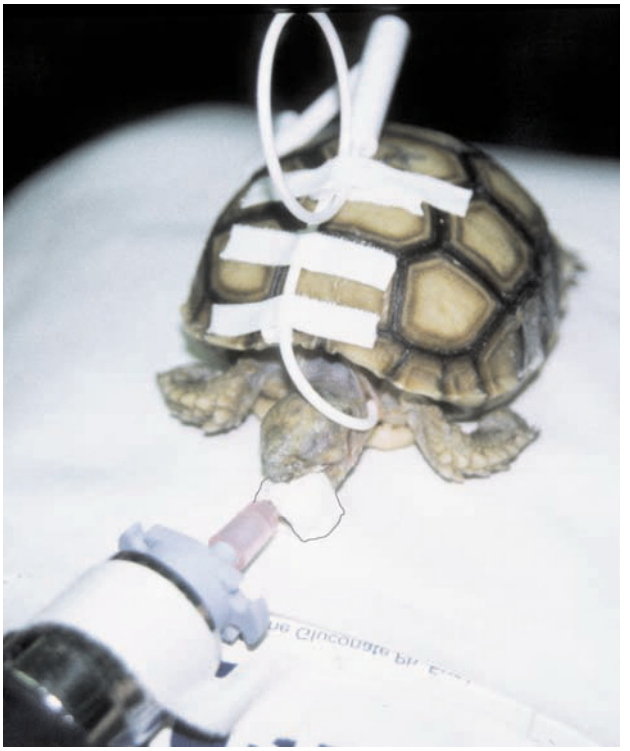

Fig. 10.20 This small hatchling *Geochelone sulcata*, weighing less than 50 g, easily tolerates a latex nasogastric tube placed as an oesophagostomy tube, in this case under general anaesthesia.

It is crucial that fine tubes like this are flushed between uses in order to reduce blocking. Any food or medication passed must be adequately liquefied. Critical Care Formula® (Vetark UK) and water-soluble medications are ideal.

Fig. 10.21 *Testudo horsfieldi*: these Horsfield's tortoises are under treatment for viral stomatitis. During recovery they are offered fresh food in addition to liquidised food administered through their oesophagostomy tubes.

Fig. 10.22 During recovery from disease the animals in Fig. 10.21 eat easily despite the presence of an oesophagostomy tube. Whilst these tubes are no longer needed to provide nutrition, they are being maintained for the administration of medications and fluids.

of oesophagostomy tubes are ideally suited to the management of infectious diseases in situations where supportive nursing and medication of 20–30 animals or so would otherwise be impossible due to lack of staff.

There is discussion in the literature concerning the size and type of patients suited to receiving an oesophagostomy tube, for example Bonner (2000) suggests that small and semi-aquatic chelonians may not tolerate O-tubes very well. However, this is not the experience of this author (SM). Here I try to illustrate the use of oesophagostomy tubes in a wide variety of situations: including small hatchlings of less than 100 g where repeated access to the mouth is unrealistic; seriously debilitated and collapsed terrestrial chelonians suffering from upper digestive tract disease; semi-aquatic species that only feed underwater with lesions requiring that they are prevented from immersion in water; animals with severe damage to the head, jaw and associated structures and large animals with the strength and determination to prevent access to their heads without the use of chemical restraint.

Occasional veterinary texts suggest that animals require forelimbs to be immobilised using tapes and bandages to prevent tube removal, however this is seldom necessary if the tube is adequately secured and protected. I have tried to illustrate a variety of ways in which the nuchal scute and carapace can be used to secure a flexible O-tube preventing the patient from being able to interfere with it. Overall, oesophagostomy tubes are well tolerated by most chelonians and are crucial to the management of most animals requiring intensive long-term rehabilitation.

Equipment

Equipment used by this author (SM):

- nasogastric (NG) tubing (e.g. Portex Feeding Tube, Infant 8F), cut to about a plastron length with the female mount preserved);
- long-handled crocodile forceps or haemostats;
- scalpel blade;
- suture material (e.g. PDS II® Ethicon, Johnson & Johnson International);
- surgical tape (e.g. Durapore®, 3M).

This author tends to use an NG tube of the largest gauge tolerable, even in smaller patients, and is aware of colleagues using lamb NG feeding tubes.

Placement

Some animals, especially large or aggressive specimens, may require sedation or even general anaesthesia. Low dose IV ketamine, in combination with local anaesthesia and non-steroidal anti-inflammatory drugs, as described in the anaesthesia section of this book, suit the majority of cases. If general anaesthesia has not been undertaken, local analgesia should be considered for the area of skin to be incised.

(1) Pre-measuring and cutting the tube is helpful. The preferred length will depend upon the method used to secure it. This author lays the end of the tube in the middle of the carapace and measures it around the cranial carapacial inlet and across the plastron to the junction of the humeral and pectoral scutes.
(2) Mark the tube to half the plastron length. Lubricate it. Before passing a stomach tube always ensure that it is filled with liquid and not air.
(3) Extract the head and stabilise the animal with the help of an assistant as already shown earlier in this book with respect to venepuncture. If the chelonian is easy to handle, the operator can hold its head and neck in extension with the patient facing towards them, whilst an assistant supports the weight of the animal. If uncooperative, the head can often be drawn out using gentle traction from a blunt dental spike hooked under the upper beak.
(4) Standing on the opposite side of the table from your assistant, open the chelonian's mouth.
(5) Insert long-handled crocodile forceps through the mouth into the oesophagus with the neck held in extension.
(6) Push the forceps laterally to protrude in the mid to distal $^1/_3$ of the oesophageal region.
(7) Cut down horizontally over the protruding tips of the forceps using a scalpel, and push the tips through the oesophageal wall and skin.
(8) If the dorsal and ventral jugulars, carotid or lymphatic vessels are visible avoid them. In the unlikely event of hitting one of the above vessels, apply temporary pressure with a swab or plug of cotton wool or place a haemostatic mattress suture.
(9) Pick up the end of the tube using the forceps and draw it in through the skin, up the oesophagus and out of the mouth by withdrawing the forceps.
(10) Reverse the grip on the tube and take it back down the oesophagus until it is in the region of the stomach. This is easy to feel if you are gentle.
(11) Put a finger suture around the tube and anchor it to the skin of the neck. Alternatively, withdraw the tube a little, stick on a tape butterfly and put two mattress sutures through the tape and skin of the neck to anchor it.
(12) Apply a small strip of tape to the external end of the tube and tape it to the nuchal scute of the carapace. Adequate nuchal-scute anchorage prevents self-removal. Carry on taping with more strips.

Animals that repeatedly remove their tubes can have them anchored in a watch spring fashion to the skin of the dorsal neck and nuchal area in order to restrict leg access. The author finds this more suitable than taping front legs back as suggested in some texts.

Tube care

Always flush with water after use or the tube may block. It may be left capped and filled with clean water between uses. Tubes are often tolerated for three to six weeks in debilitated animals. Occasionally they can remain *in situ* for three months or more. Provided care is taken to observe for infections, complications are rare. Once an animal has recovered well enough to eat around an oesophagostomy tube it is common for them to remove it suddenly, where previously it had been well tolerated.

Removal

Removal is relatively straightforward and often involves only simple restraint by an assistant on the other side of an examination table. Any butterfly tape or finger sutures retaining the tube are released. The tube can then be withdrawn through the incision, tape removed and all materials discarded. The portion of the tube within the lumen of the oesophagus collects all sorts of foul material, and if removed in front of a client it may be best to warn them about this before they see and smell it. Where an animal is already on long-term antibiosis, further antibiotics are not necessary. Where an animal is not receiving antibiotic cover the wound should be carefully checked over the following three to six weeks for any evidence of infection. Complications are unusual.

Semi-aquatic species

Semi-aquatic species also tolerate oesophagostomy tubes, which are easily anchored by placing a blob of superglue or epoxy on the taping sites (Figs 10.23–10.26). These animals may require reduced water exposure. The need for reduced water exposure in the management of some conditions is a primary indication for the placement of an oesophagostomy tube as many species will only feed underwater.

Fig. 10.23 Semi aquatic specimens also tolerate such tubes, which are easily anchored by placing a blob of Superglue® or epoxy on the taping sites. These animals may require reduced water exposure.

The need for reduced water exposure in the management of some conditions is a primary indication for the placement of an oesophagostomy tube as many species will only feed underwater.

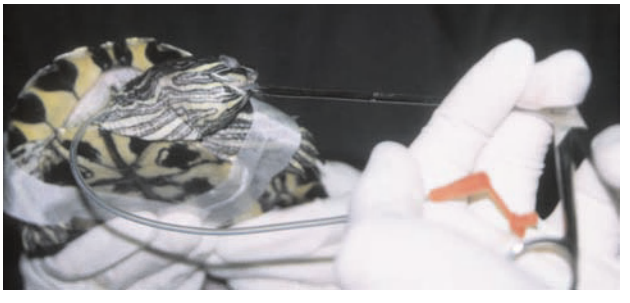

Fig. 10.24 Tape can be used to restrain the turtle. Analgesia and sedation are generally required for placement.

Fig. 10.25 Application of Superglue® (Loctite) to zinc oxide tape fixings allows the animal to immerse itself in water. The tape and glue will need maintenance every three or four days.

Fig. 10.26 Alternative tape and tube anchorage beneath the nuchal scute in the red-reared slider (*Trachemys scripta elegans*).

Here the tube has been secured to reduce possible removal by the animal's long and strong front claws.

This animal has a large facial abscess.

FLUID MANAGEMENT

In chronically-ill terrestrial chelonians, those receiving medications or those undergoing anaesthesia it is essential to maintain adequate fluid balance.

Most unwell terrestrial chelonians benefit from oral fluids. Fluids should be appropriately warmed before administration.

Table 10.2 summarises the use of fluids for maintenance and to correct imbalances.

ROUTES OF FLUID ADMINSTRATION

Sites and techniques for fluid therapy and feeding include (Figs 10.5–10.8 & 10.27–10.39):

- oral fluids;
- fluids by stomach tube;
- fluids by oesophagostomy tube;

Table 10.2 Fluid management in maintenance and correction of imbalances.

Maintenance	• Consider the use of all or any routes described later. Species, ease of handling and condition will all influence the choice of route. • Shallow bathing, once or twice a day, allows rehydration and also helps stimulate urination, which appears reflex upon bathing in many species. • Maintenance volumes for individual species sizes, sex and ages are not yet available and so we are forced to consider much anecdotal advice. Suggested rates are given later.
Dehydration and hypovolaemia	• Dehydration and its assessment are covered in detail elsewhere in this text (see Clinical Pathology). • The volume of fluid present within the bladder, in conjunction with the role of the chelonian bladder as a fluid reservoir and detoxifying organ, complicates biochemical and physical assessment of dehydration. • Advice available regarding how to rectify dehydration in a chelonian is subject to a great deal of opinion and anecdotal comment.

Fig. 10.27 Regular bathing encourages reflex urination and defecation. Following urination some chelonians are able to at least partially refill their bladder with water from the bath. This may then be reabsorbed across the bladder, aiding rehydration.

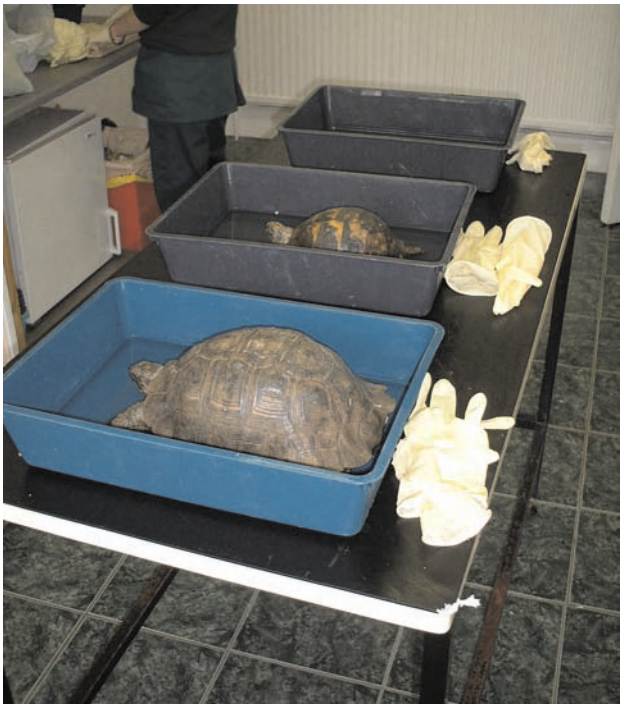

Fig. 10.28 At this author's clinic (SM) all hospitalised chelonians are provided with a warm daily soak of about 20 minutes' duration prior to feeding. Bowls may be placed on top of a heating mat to preserve the temperature during bathing.

Fig. 10.29 Intracoelomic fluids are administered into the prefemoral fossa. This route carries the risk of puncture of ovarian follicles, in mature females, or the bladder. Excessive administration of fluid may result in respiratory compromise. This author (SM) prefers the epicoelomic route to the intracoelomic route.

- intracoelomic fluid injection;
- epicoelomic fluid injection;
- intraosseous fluid administration;
- intravenous fluid administration;
- bathing and cloacal fluids;
- subcutaneous fluids.

Oral fluids

Animals can be encouraged to drink by regular bathing in warm water, which is often drunk during soaks. During bathing the

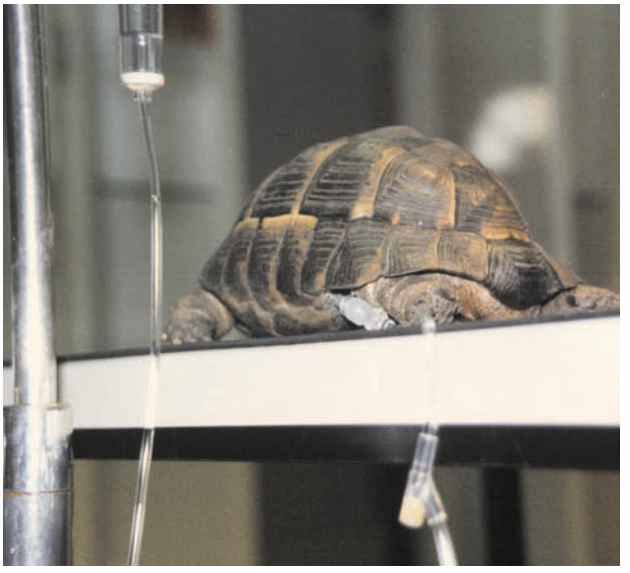

Fig. 10.30 A paediatric burette and giving set can be used to give controlled-rate, limited-volume infusions into the coelomic cavity.

Fig. 10.31 The epicoelomic route is greatly favoured by this author (SM). One percent of the animal's body weight is well tolerated and can be administered relatively quickly without distress to the animal or effort from the operator. The site is the potential space between the plastron and the pectoral musculature.

whole of a tortoise's head may be submerged beneath the water for a considerable length of time and animals may take water in through the nares as well as the mouth.

Fluids can be provided to herbivores by offering a high proportion of succulent foods such as melon and cucumber.

Syringes can be used to squirt liquidised food and water into chelonians' mouths (Fig. 10.4) but this is relatively ineffective in comparison to passing a stomach tube. In addition, the size, anatomy and strength of chelonians will limit the use of such a technique.

Many keepers suggest that bread soaked in water encourages a high fluid intake to post-hibernation tortoises. Whilst such a diet would appear relatively unnatural and is not encouraged by this author (SM), no obvious adverse effects have been seen following its use in this situation by keepers attending my surgery.

Fig. 10.32 Following pre-treatment blood sampling, most animals admitted to this author's ward (SM) are given 0.5%–1% of their bodyweight by the epicoelomic route. If animals have a history of water deprivation, e.g. in the post-hibernation period, 1%–2% body weight per day of fluids are given. This volume is divided between the oral and epicoelomic routes and given in small amounts throughout the day.

Fig. 10.33 Here an intraosseous needle has been inserted in the tissue of the bridge and tape is used to retain it in place.

It is not firmly established that fluid given by this route is readily absorbed and many syringe drivers and needles seem to obstruct, especially in older animals with heavily-ossified shells.

Alternative sites for intraosseous fluids or medications are the femur or humerus.

Fig. 10.34 Here a juvenile hingeback tortoise (*kinixys erosa*) is connected to an intraosseous drip, resistance tubing and a spring-loaded syringe driver (Flowline®, Animalcare, UK). Other equipment illustrated includes a gag and gavage tube containing Critical Care Formula® (Vetark, UK).

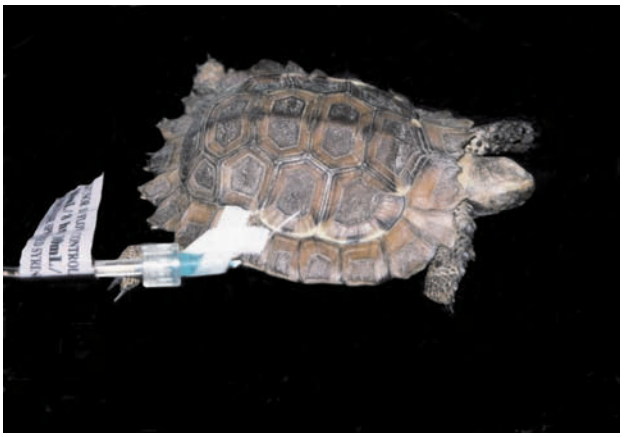

Fig. 10.35 A close-up of the animal in Fig. 10.34, showing Flowline and needle insertion in the bridge of the shell.

Fig. 10.36 Intraosseous and intravenous fluids can be controlled by flow regulators such as this Vet/IV 2.2® (Heska, Fort Collins, USA).

Fig. 10.37 *Testudo graeca* attached to an intraosseous drip, spring-loaded syringe driver and Flowline tube®.

Fig. 10.38 The bladder is relatively easily catheterised, using a speculum and Foley catheter, following the technique described by Dantzler & Schmidt-Nielson (1966). This allows the administration of fluids and medications directly into the bladder and the removal of urate deposits by lavage.

Fluids by stomach tube (gavage)

Delivery by the gavage seems to be an excellent method of stabilising and supporting a debilitated chelonian, provided they are easily handled and do not become excessively stressed by this procedure (Figs 10.5–10.8). Fluids suited to oral administration are discussed later in this section, and are best given divided into three or more amounts distributed throughout the day.

Most chelonians will tolerate 0.5%–2% body weight per day in divided doses for maintenance without complications. Higher rates of administration, e.g. 2%–4% of water per day, suit stabilisation during periods of significant dehydration such as in the post-hibernation, anuric, hyperkalaemic and hyperuricaemic chelonian. Four per cent body weight per day is only required until fluid balance is normalised and urination achieved. Fluid administration

Fig. 10.39 Coelomic dialysis is described as a management technique for the treatment of hyperuricaemia, however it seems to offer little advantage over traditional administration of fluids or bladder catheterisation.

levels can be reduced gradually to 1% then 0.5% of bodyweight per day as urination is achieved.

Water is an ideal replacement for cases of hypertonic dehydration. It can then be altered to a nutritional support and electrolyte solution as fluid balance is normalised and urine output achieved.

Fluids by oesophagostomy tube

The same points made with respect to fluids given by oral gavage/stomach tube generally apply to fluids given by oesophagostomy tube. Oesophagostomy tube placement suits animals likely to require medium- to long-term nutritional, fluid or oral medical support.

Oesophagostomy tube placement is especially suited to recalcitrant species, such as *Geochelone pardalis* and *Testudo horsfieldi*. It is impractical to manually extend the head and open the beak to pass a stomach tube on a regular basis in such animals without either spending an inordinate amount of time or causing the animal a great deal of distress. Similarly, aggressive species are easily medicated by such a technique. Oesophagostomy tubes have even be used with some success in marine turtles.

Oesophagostomy tube placement is illustrated in Figs 10.9–10.20.

Epicoelomic fluid injection

Fluids may be administered by injection through the cranial inlet of the shell, laterodorsal to the head and neck, immediately dorsal to the plastron and below/into the pectoral muscles (Figs 10.31, 10.32). The author (SM) uses this site regularly.

It is possible that this area connects to the pericardium and other natural fluid-storage sites making it ideally suited to fluid administration. Fluids seem rapidly absorbed without obvious complications and animals can accept 1%–2% of body weight divided into small amounts and given several times throughout the day on a regular basis. This route suits critically-dehydrated patients, for example those displaying anuria, sunken eyes and decreased skin elasticity.

Ease and speed of handling make the epicoelomic route ideal for most hospitalised chelonians unable to maintain their own fluid balance without intervention. Handling is limited and fluids can be administered with the head and neck flexed or extended, and such a technique is easily explained to inexperienced clinicians seeking telephone advice regarding the stabilisation of cases.

In this author's experience (SM), absorption of fluid and circulatory support seem more likely to be guaranteed by administration of fluids by this route compared with the intraosseous or intracoelomic administration advised in many earlier texts. Potential complications of other routes are described in the relevant sections.

Intracoelomic fluid injection

Fluids may be injected into the coelomic cavity through a surgically-prepared site in the prefemoral fossa (Fig. 10.30). This author tends to favour the epicoelomic route over the coelomic route as fluids are not guaranteed to be absorbed at any useful speed from the coelomic cavity. The mechanisms involved in fluid adsorption from the bladder are not adapted to absorb fluid from serosal surfaces.

In our experience, up to 3% of body weight per day in divided doses can be given safely, but care should be taken to avoid trauma to the bladder or compression of the respiratory space.

Mader *et al.* (2002) describe a technique for surgically placing a semi-permanent, intracoelomic, 14–18F silicone catheter with access port, into sea turtles through the prefemoral fossa. This tube can be used to provide parenteral fluids and medications for up to five days.

Intraosseous fluids

Intraosseous fluid administration is popular in many veterinary texts (Figs 10.33–10.37), but in this author's (SM) experience is often complicated by a failure of syringe driving equipment or needle blockage. Little benefit is gained by an animal connected to a syringe driver for several hours, in a critical state, if fluid isn't actually going in! Unfortunately, it is often only the passage of time that tells the clinician that this is the case.

Intraosseous fluids are better given in patients without heavily calcified shells. Examples of these include juvenile terrestrial tortoises, softer, proteinaceous-shelled species such as box turtles (*Terrapene* and *Cuora* spp.) and semi-aquatic species such as red-eared sliders (*Trachemys* spp.).

It is arguable that in mature *Testudo* species it is necessary to drill a pilot hole in the shell before a needle can be inserted. The usual site is called the bridge and this is the junction of the carapace and plastron cranial to the hind limb. Other sites used include the costal/marginal scute junction in softer-shelled animals and the limb bones. As the technique is unreliable and often takes a considerable time to resolve a circulatory problem, this author generally prefers administration of fluids by the epicoelomic route.

Intravenous fluids

Intravenous fluids are suitable only for severely debilitated chelonians requiring emergency treatment, or anaesthetised animals,

as a jugular cut down is required (Figs 6.42–6.44). Some authors have administered colloid blood replacements and blood transfusions intravenously, but their efficacy is currently unknown and unproven.

The route is also available for intravenous medications.

Bathing and cloacal fluids (lower urinary tract absorption)

The chelonian bladder has a novel role of fluid absorption and is part of the animal's mechanism to conserve and recycle renally-derived fluids. In giving fluids by this route the clinician is utilising the optimum route for fluid uptake in many species of chelonians (especially predominantly uricotelic species). The physiology of such animals suggests that fluids given by such a route are likely to be absorbed rapidly and taken promptly into the circulation.

All hospitalised chelonians at the author's clinic are bathed in shallow, warm water once or twice daily for ten minutes throughout the duration of their rehabilitation (Figs 10.27–10.28). This appears to stimulate reflex urination and defecation. It is proposed that fluids are drawn into the cloaca where absorption through the cloaca, bladder or colon is possible. Observing most *Testudo* spp. in baths, it is possible to see movement of water within the bath as it is drawn into and flushed out of the cloaca as the chelonian moves its limbs and alters its intracoelomic pressure.

In severely hyperuricaemic patients, fluids may be actively flushed into the cloaca and bladder, removing lower urinary tract toxic excretion products and facilitating lower urinary tract fluid absorption. Bladder catheterisation, using a large bore Foley catheter, allows bladder lavage and dialysis in such cases. Urine output and removal of more solid urate precipitates must not be prevented in such animals or their condition may actually worsen.

Subcutaneous fluids

Subcutaneous fluids can be administered in the prefemoral fossa and between the neck and the front limb. A small-gauge needle should be used and the needle inserted as far dorsally as possible, in order to avoid leaking of the fluid from the injection site. The animal should be kept at its ATR to assure good peripheral perfusion allowing good resorption and several deposits can be made at any one time.

OVER-HYDRATION

A genuine risk of tissue oedema exists if non-oral routes are used to administer excessive fluid in the presence of renal, cardiac or respiratory disease. Tissue oedema is more likely with systemic administration of hypotonic and isotonic fluids.

It is impossible accurately to quantify fluid deficits. The physical signs of emaciation and generalised illness and debility mimic those of dehydration. There is therefore more of a danger of inducing over-hydration in reptiles than in mammals. It may be hard to distinguish signs of over-hydration from those of deterioration in the condition under treatment. Most consequences of over-hydration result in non-specific depression and so are unlikely to facilitate diagnosis. Table 10.3 lists the possible consequences of over-hydration, and their effects on the patient.

Table 10.3 Consequences of over-hydration and their effects.

Cerebral oedema	Animals are likely to be depressed, anorexic and inactive and may respond poorly to visual and other stimuli.
Pulmonary oedema	As chelonians lack a diaphragm and cough reflex, it will be hard to assess and diagnose pulmonary oedema in a patient undergoing active fluid therapy. Tachypnoea may be an early sign. Dyspnoea is unlikely to be a feature until the problem has become highly advanced. It is possible in an extreme situation that the animal will breathe with its mouth open and neck extended. A nasal discharge may be present.
Ascites	Animals are likely to have gained weight rapidly in response to fluid administration and limbs and coelomic lining tissue are likely to have become visually distended. Likely to occur in combination with cerebral and pulmonary oedema.

It is important to monitor body weight, urine output and volume of fluid administered over time in order to reduce the likelihood of tissue oedema in animals receiving active fluid therapy.

FLUIDS FOR ORAL REHYDRATION

The oral route is excellent for rehydration, provided that the patient can be stomach-tubed (gavage) or has an oesophagostomy tube fitted. Potassium losses can be replaced safely. The ideal solution for many sick chelonians is unknown—no objective data are available. We routinely administer 5 ml/kg of water and liquidised vegetables twice daily with apparent success.

SYSTEMIC FLUID THERAPY

Fluids equivalent to 1%–3% of body weight can be safely administered to potentially dehydrated chelonians during initial stabilisation (SM: personal data). Wild chelonians are known to tolerate large fluctuations in blood osmolarity (Gilles-Baillien & Schoffeniels 1965).

Mammalian fluid preparations are suitable for most chelonian situations. Our experience is that many ill tortoises have isotonic or hypotonic dehydration. It is possible that lactated fluids (Hartmann's solution) should not be used, since lactic acidosis is a common problem in stressed chelonians (Prezant & Jarchow 1997). However, Mader (2001: Personal communication) suggests that, *in vivo,* lactated fluids form a physiological buffer solution and can actually reduce complications associated with acidosis.

Historical advice to administer hypotonic fluids preferentially to dehydrated reptiles based upon body fluid compartmentalisation may be inaccurate. Such a theory appears to have originated following comments by Thorson (1968a) regarding the ratio of extracellular fluid (ECF) to intracellular fluid (ICF) in chelonians. Unfortunately, Thorson's calculations placed the considerable

volume of fluid present in the chelonian bladder within the ICF body compartment. He consequently drew a conclusion that chelonians have an increased ICF/ECF ratio when compared to mammals. Upon looking at the volume of fluid contained within the bladders of normal tortoises, and then removing it from the ICF, this author (SM) has actually found that the reverse may be true and chelonians may have a reduced ICF/ECF ratio when compared to mammals. It would seem logical that hypotonic fluids may only benefit those chelonians with hypertonic dehydration.

It is best to try and assess what sort of deficit may be present and to give appropriate fluids to counteract this. Electrolyte assessments and osmolarity calculations as given in the earlier Clinical Pathology chapter assist the clinician with this. Some empirical guidelines used regularly by this author in *Testudo* spp. are given in the problem-solving sections of this book, later on.

DEHYDRATION

Overall glomerular filtration rate for the reptilian kidney tends to decrease with dehydration or salt loading and increase with water loading. There are significant differences in the magnitude of the glomerular response in chelonian species from different environments (Dantzler 1976). It is suggested that neurohypophyseal hormones, such as arginine vasopressin, may exert some control over the glomerular filtration rate. It is not clear how renal excretion of uric acid is influenced by glomerular filtration rate, as active excretion occurs. If active secretion of uric acid within the proximal tubules continues in the absence of fluid to carry it away, the urate-filled tubules and ruptured glomeruli seen in histopathological examination of end-stage kidneys would presumably result (McArthur: personal observation).

Glomerular filtration ceases when plasma osmolarity is significantly raised by about 20 mOsm above normal in some semi-aquatic chelonians such as *Trachemys scripta*, and by about 100 mOsm above normal in some terrestrial species such as *Gopherus agassizii* (Dantzler & Schmidt-Nielson 1966).

Following a normal wild hibernation or a well-managed captive hibernation, restricted fluid input before and after the hibernation results in pre-renal azotaemia (Gilles-Baillien & Schoffeniels 1965). A more clinically significant pre-renal azotaemia is often demonstrated in chronically-ill chelonians as a result of chronic dehydration, excessively long hibernation periods, protein catabolism and poor fluid management, especially in the post-hibernation period.

According to Lawrence (1987b), a poorly-managed chelonian hibernation predisposes to terminal renal failure. Lawrence suggests that an increase in plasma osmolarity of greater than 100 mOsm, as described earlier, can easily occur during prolonged post-hibernation anorexia. He suggests the physiological cessation of urine production in such animals is likely to be fatal if not treated rapidly with fluid administration. Indeed, renal deposition of urates (gout), hyperkalaemia and death are likely if rehydration is not achieved relatively quickly. A normal or well-managed hibernation should result in a moderate pre-renal azotaemia that is rapidly reversible in the post-hibernation period (Gilles-Baillien & Schoffeniels 1965) (Table 10.4). In the absence of stressors, this should not be clinically significant.

SIGNS OF DEHYDRATION/ HYPOVOLAEMIA

In most chelonians, dehydration is extremely hard for a clinician to assess until it has become well established. This is because of the array of compensatory homeostatic mechanisms that chelonians have available to buffer the physiological impact of water loss. Recovery of fluid from the lower urinary tract and large intestine to rehydrate/stabilise body tissues during long periods of water deprivation, are very important methods of countering dehydration for terrestrial chelonians (Bentley 1962; Jorgensen 1998). Evaporative water loss in these species is restricted.

No physical signs are specific to dehydration and all those listed below could just as easily be considered to be signs of emaciation and ill health generally. It would seem that chelonian dehydration is not easily recognised whilst reptilian clinical pathology is still in its infancy.

Dehydrated chelonians may demonstrate few physical changes during moderate compensated dehydration:

Table 10.4 Seasonal changes in electrolyte and urea concentrations in healthy, winter-hibernated *Testudo hermanni* (Gilles-Baillien & Schoffeniels 1965).

	Na^+ mEq/l	K^+ mEq/l	Ca^{++} mEq/l	Cl^- mEq/l	Urea mM/l	Osmotic Pressure mOsm/l
Jan	156	3.7	2.4	124	31	349
Feb	161	3.0	5.3	123	38	449
March	157	3.8	5.4	125	34	443
April	167	4.6	4.6	134	103	467
May	129	5.0	4.9	86	37	340
June	105	4.3	1.5 (sic)	66	26	258
July	115	4.5	4.5	94	4	290
Aug	136	4.8	5.5	108	12	322
Sept	136	4.1	4.9	99	11	338
Oct	138	4.2	4.8	110	22	343
Nov	141	3.0	5.2	99	21	349
Dec	155	3.9	6.3	124	31	404

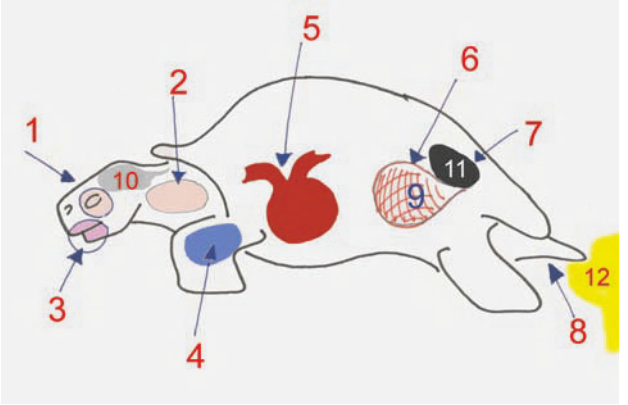

Fig. 10.40 Dehydration in a uricotelic terrestrial chelonian
(1) Sunken eyes
(2) Decreased skin elasticity
(3) Dry, tacky, mucous membranes
(4) Muscular weakness
(5) Altered plasma biochemistry
(6) Precipitation, if urate within the bladder is increased
(7) Loss of glomerular filtration
(8) Decreased urine output
(9) Exhaustion of bladder fluid reserve
(10) Gouty deposits within the brain
(11) Gouty deposits within the kidneys and other visceral organs such the heart and joints
(12) Alterations in urine specific gravity (SG) (increased) and pH (decreased)

- decreased body weight;
- decreased activity;
- decreased frequency and quantity of urination;
- increased urine specific gravity.

Specific gravity is normally 1.003–1.012 in *Testudo/Gopherus/Terrapene* spp., and 1.008–1.017 in healthy animals at the end of hibernation or drought (Innis 1997b; Christopher *et al.* 1994; Gibbons 2000; McArthur & Wilkinson: personal observations). Although the kidneys invariably produce hypo- or isosmotic ureteral urine, resorption from the lower urinary tract results in some concentration.

As the animal becomes more severely dehydrated and decompensated, additional observations may include (Fig. 10.40):

- sunken deflated eyes (bilateral enophthalmos);
- decreased skin elasticity, deflated limb structure, deflation of temporal musculature in extreme cases;
- decreased salt gland activity (marine reptiles).

BIOCHEMICAL CHANGES (URICOTELIC SPECIES)

With moderate, compensated dehydration, fluid within the bladder is used to buffer physiological changes, and no change other than increased urine specific gravity may be noted.

In severe decompensated dehydration, fluid reserves in the bladder are absent or their ability to buffer physiological changes have been exhausted, and further increased urine specific gravity, hyperuricaemia, uraemia, increased haematocrit and albumin levels, acidosis, hyperkalaemia and visceral and articular gout may occur.

Table 10.5 Complications in assessing blood biochemistry (Wilkinson 2000).

Increased plasma uric acid	Probably a relatively insensitive indicator of dehydration, particularly in species from arid environments, where renal urate excretion is relatively resistant to rises in plasma osmolarity. High uric acid is indicative of renal dysfunction; dehydration may or may not be present.
Increased plasma urea	Terrestrial tortoises do not normally pass urine during periods of water shortage. Rather, bladder contents are retained in order that water and electrolytes may be resorbed across the bladder wall. Urea is a small molecule, which readily crosses biological membranes and also re-enters circulation in these circumstances. Azotaemia is a normal feature of hibernating tortoises (up to 100 mmol/l in Mediterranean *Testudo* spp.) and may be a useful indicator of dehydration. Azotaemia plus hyperuricaemia is more often consistent with renal failure.
Increased haematocrit	Uraemia is probably common in sick tortoises. Profound seasonal variations may occur. 'Normal' data may be unavailable for many species.
Increased plasma albumin	Hypoalbuminaemia is common in sick animals. Hyperalbuminaemia is usual in summer in females of temperate species.

Table 10.5 sets out some of the complications and factors which must be borne in mind when assessing chelonian dehydration through blood biochemistry.

It would appear likely from the data describing the seasonal changes in electrolyte and urea concentrations in healthy, winter-hibernated *Testudo hermanni* (Table 10.4), that April elevations to 288 mg/dl (103 mmol/l) are the result of post-hibernation catabolism in combination with normal post-hibernation dehydration. Normal values three months later were only 11.2 mg/dl (4 mmol/l). The work of these authors also demonstrates a temporary elevation in potassium as urea and plasma osmolarity levels drop.

The use of urea values as a method of assessing renal function in reptiles is now regarded as being of limited value (Campbell 1996b; Mader 1997; Divers 2000b). It is likely that urea values quoted by Lawrence (1987b) reflect renal pathology or terminal biochemical derangements in this particular situation. However, significant elevations in urea values should be treated with a high degree of clinical suspicion in both aminoureotelic and ureouricotelic species. Uraemia may indicate renal failure, dehydration, catabolism, excessive dietary protein or any combination of the above. It is hard to distinguish between these causes, as normal levels are poorly defined.

INTERPRETATION OF PRESENTING SIGNS

STUART MCARTHUR

The following is given as an aid to clinicians in compiling a differential diagnosis list. It is not intended to be exhaustive, but rather to complement the thoughts of the clinician. It is meant to be used alongside the anamnesis, imaging and clinical pathology techniques and detailed physical examination in order to determine the likely diagnoses and to make an appropriate care plan. Figures 11.1–11.105 accompany the text.

EMACIATION

See Anorexia below.

ANOREXIA

- Any acute or chronic disease
    - Gastrointestinal disease (foreign body/impaction/parasitism)
    - Respiratory disease
    - Nutritional disease
    - Metabolic disease (e.g. hypocalcaemia, dehydration, ketoacidosis, azotaemia)
    - Follicular stasis/egg retention
    - Renal failure
- Sight impairment (frost damage, intraocular disease, central nervous system disease, etc.)
- Inappropriate environmental provision (too cold, too dark, too humid, too dry, too hot, etc.)
- Inappropriate food provision (meat to a herbivore, lettuce to an omnivore etc.)
- Maladaptation (e.g. sudden environment change in captivity following wild capture)
- Social disruption (e.g. loss of vivarium mate, intra- or inter-species aggression)
- Psychological (e.g. post physical trauma, transportation stress)
- Advanced folliculogenesis in breedable/reproductively active females (normal)
- Aestivation in normal animals in good weather (especially *Testudo horsfieldi*) (normal)
- Behavioural (e.g. male intent on mating or female during ovulation/oviposition) (normal)

INACTIVITY/LETHARGY

- Inappropriate temperature provision (too cold, too hot etc.)
- Inappropriate environmental provision (too humid, too dry, too bright, too dark, etc.)
- Intra- or inter-species aggression (e.g. housing with incompatible animals)
- Maladaptation of captive wild-caught specimen
- Any acute or chronic disease
    - Gastrointestinal disease (foreign body/impaction/parasitism)
    - Respiratory disease
    - Nutritional disease
    - Metabolic disease (e.g. hypocalcaemia, dehydration, ketoacidosis, azotaemia)
    - Follicular stasis/egg retention
    - Renal failure
- Gravidity
- Behavioural distress
- Sight impairment
- Starvation, hypoglycaemia, other metabolic derangement
- Inappropriate hibernation management (before, during or after hibernation)
- See also Ataxia, Generalised Weakness and Paresis

GENERALISED WEAKNESS

- Hypocalcaemia
- Hypoglycaemia/starvation/ketoacidosis
- Septicaemia
- Metabolic bone disease
- Other nutritional deficiencies
- Dehydration
- Over-hydration
- Intoxication (e.g. endotoxins, putrefaction of ingesta, pesticide contamination of food, inappropriate drugs/dosages)
- Heat stroke
- Frost damage
- Any chronic or acute disease

EXCESSIVE WEIGHT GAIN

- Inappropriate nutrition (e.g. dog or cat food to herbivorous species)
- Oedema
- Over-hydration
- Renal failure
- Gravidity
- Dystocia
- Cystic calculi
- Follicular stasis
- Obesity
- Congestive heart failure
- Metabolic disease (e.g. hypothyroidism or similar endocrinopathy)
- Hepatic lipidosis

UNDERWEIGHT

(significant, unexplained weight loss)

- Anything predisposing to chronic anorexia
- Any acute or chronic disease process
- Inappropriate environmental provision
- Inappropriate dietary provision
- Starvation
- Maladaptation
- Dehydration (e.g. excessive renal or intestinal loss of fluid)

PARESIS

(generalised or hind limb)

- Neuropathies secondary to endocrinopathies such as hyperparathyroidism or hyperoestrogenism (i.e. follicular stasis)
- Ketoacidosis
- Hypocalcaemia
- Egg retention
- Renal enlargement
- Constipation
- Urolithiasis
- Septicaemia
- Spinal infection (discospondylitis/osteomyelitis)
- Central nervous system: infection/meningitis
    - Visceral *Larva migrans*
    - Viral infection
    - Protozoan infection
    - Bacterial infection
    - Mycotic infection
- Intoxication
    - Failure to remove pesticide residues on food
    - Putrefaction of ingesta as a result of inadequate temperature provision
    - Failure to starve before hibernation
    - Inappropriate medication (e.g. Ivermectin administration)
- Carapacial/spinal trauma
    - Frost damage/heat damage
    - Lawnmower strike (terrestrial)
    - Speedboat strike (marine)
    - Neuropraxis
- See also Generalised Weakness

ATAXIA, CONVULSION, CIRCLING

- Head trauma/frost damage/heat trauma
- Otitis media/ear abscessation
- Hepatoencephalopathy
- Central nervous system: infection/meningitis
    - Visceral *Larva migrans*
    - Viral infection
    - Protozoan infection
    - Bacterial infection
    - Mycotic infection
- Metabolic disease (e.g. hypocalcaemia, hypoglycaemia, ketoacidosis)
- Toxicity (e.g. pesticide exposure, inadequate washing of purchased fruit and vegetables)
- Hypovitaminosis B1 (excess of frozen fish in diet)
- Inappropriate drug use/dosage (e.g. Ivermectin toxicity)

ABNORMAL MUCOUS MEMBRANE COLOUR

- Hepatic disease, jaundice, biliverdinaemia
- Respiratory disease
- Cardiac disease
- Anaemia
- Septicaemia
- Toxaemia
- Localised infection
- Stomatitis
- Lymphoproliferative disease
- See also Apparent Anaemia

APPARENT ANAEMIA

- Blood loss
    - Haemorrhage
    - Excessive sample size during phlebotomy
- Lymphodilution of blood sample
- Renal disease
- Respiratory disease
- Cardiac disease, circulatory failure
- Chronic disease, starvation
- Parasites
- Neoplasia
- Bone marrow suppression (e.g. iatrogenic, endocrinological or metabolic)
- Haemolytic diseases
- Toxins
- Nutritional mismanagement

MUCOUS MEMBRANE PALLOR

- Circulatory disease
- Cardiac disease
- Inappropriate temperature provision (too cold)
- Respiratory disease
- Hibernation
- See also Apparent Anaemia

JAUNDICE

- Hepatic disease
- Post-hepatic obstruction
- Haemolytic disease
- Common in the post-hibernation period, especially where husbandry provision and management are poor.

ABNORMAL FLOTATION

- Respiratory disease
- Gastrointestinal disease (e.g. bloat/fermentation, foreign body, obstruction)
- Free air in the coelomic cavity from respiratory or intestinal leakage or microbial fermentation

- Gravidity/dystocia
- Coelomic lesions (fibropapillomas, uroliths etc.)

POST-HIBERNATION ANOREXIA

- Often the result of chronic disease being exacerbated during a period of torpor
- Inappropriate pre- or post-hibernation management (e.g. inadequate/inappropriate temperature, humidity or light provision)
- Prolonged dehydration/azotaemia
- Inadequate pre-hibernation starvation and secondary putrefaction of gut contents
- Post-hibernation hypoglycaemia
- Excessively long hibernation
- Frost damage/sight impairment
- See also Anorexia

BLEPHAROEDEMA

- Keratitis, conjunctivitis, ocular infections
  - Chlamydial
  - Bacterial
  - Mycotic
  - Mycoplasmal
  - Viral
- Retrobulbar infection
- Ocular foreign bodies
- Dry eye
- Generalised oedema
  - Hypoproteinaemia (hepatopathy, nephropathy, enteropathy)
  - Congestive heart failure
  - Excessive fluid therapy
- Conjunctival hyperplasia
  - Vitamin A deficiency

BLEPHAROSPASM

- Keratitis, conjunctivitis, ocular infections
  - Chlamydial
  - Bacterial
  - Mycotic
  - Mycoplasmal
  - Viral
- Retrobulbar infection
- Ocular foreign bodies
- Dry eye
- Generalised oedema
  - Hypoproteinaemia
  - Renal failure
  - Congestive heart failure
  - Lymphatic obstruction
- Conjunctival hyperplasia
  - Vitamin A deficiency
- Trauma

CORNEAL LESIONS

- Keratitis, infection
  - Chlamydial
  - Bacterial
  - Mycotic
  - Mycoplasmal
  - Viral
- Corneal trauma
- Lipidosis
- Scar tissue
- Frost damage during hibernation

BLINDNESS

- Frost damage
- Metabolic disease
- Conjunctival hyperplasia, hypovitaminosis A
- Intoxication
- Keratitis, conjunctivitis, ocular infections
  - Chlamydial
  - Bacterial
  - Mycotic
  - Mycoplasmal
  - Viral
- Retrobulbar infection
- Ocular foreign bodies
- Dry eye
- Head trauma
- Central nervous system disease

OCULAR DISCHARGE

- Dacrocystitis, panophthalmitis (haematogenous or traumatic/penetrative), keratitis, conjunctivitis, ocular infections:
  - Chlamydial
  - Bacterial
  - Mycotic
  - Mycoplasmal
  - Viral

NASAL DISCHARGE

- Upper respiratory tract infection
  - Viral infection
  - Mycoplasmosis
  - Bacterial infection
  - Mycotic infection
  - Chlamydiosis
- Metabolic disturbances
- Excessive salivation
- Inappropriate humidity and temperature provision
  - Excessive heat exposure/heat stroke
  - Vivarium too cold
  - Vivarium too humid
  - Vivarium too dry
- Exposure to mechanical/chemical irritants
- Poor vivarium hygiene
- Nutritional disease (e.g. hypovitaminosis A)
- Systemic illness
- Pain response (possible)
- Upper respiratory or buccal/oesophageal obstruction
- Trauma
- Neoplasia

DYSPNOEA

(laboured breathing/open mouthed breathing)

- Upper or lower respiratory tract infection
  - Viral infection
  - Bacterial infection
  - Chlamydiosis
  - Mycoplasmosis
  - Mycotic infection
- Parasitic migrations
- Nutritional disease (e.g. hypovitaminosis A)
- Trauma
- Aspiration pneumonia
- Inhalation of irritants
- Heat stroke/excessive heat exposure
- Neoplasia

EXCESSIVE EXTENSION OF NECK

- Lower respiratory tract disease
- Oesophageal obstruction
- Neurological disease
- Hypocalcaemia
- Generalised weakness
- Aggressive response to being approached

STOMATITIS

- Immunosuppression of any aetiology
- A potential consequence of post-hibernation leucopaenia
- Nutritional disease (e.g. hypovitaminosis A or C)
- Metabolic disease (e.g. uraemia/hyperuricaemia of renal failure)
- Infection
  - Viral disease
  - Bacterial disease
  - Mycotic infection
- Hibernating recently-fed animals, putrefaction of food remnants
- Penetration injuries from food items
- Local irritation (e.g. drinking caustic fluid, eating chemically treated food)

PHARYNGEAL OEDEMA

- Usually secondary to stomatitis
- In the author's experience, such cases are either severe or have suffered extensive secondary bacterial/mycotic infection

EXCESSIVE SALIVATION

- Stomatitis
- Oesophageal foreign body
- Upper digestive tract lesions
- Excessive heat exposure
- Neurological disease
- Intoxication/toxicosis

VOMITING/REGURGITATION

- Stomatitis, pharyngitis, oesophagitis
- Intussusception, foreign body
- Gastritis (e.g. *Cryptosporidium*, gastric tumour)
- Parasitism (e.g. helminthiasis, cryptosporidiosis)
- Iatrogenic (drug/medication reaction)
- Septicaemia/toxaemia
- Inappropriate gastric gavage technique or food
- Excessive post-prandial handling

GASTROLITHS

- The primary function of gastroliths is suggested to be digestive and most probably mechanical. In some situations lithophagy appears to be behaviourally and possibly physiologically driven.
- Situations such as vitellogenesis appear to be associated with ingestion of white material such as bone, small white stones and broken china and this would appear to coincide with increased metabolic demands for calcium.
- Disease associated with gastroliths mainly involves impaction of the large intestine as a result of ingestion of a substrate such as gravel.
- Foreign bodies associated with disease such as intestinal obstruction are rare but have been described.

DIARRHOEA

- Normal—the product of the normal excretion of renally derived fluids mixed with faeces
- Unsuitable/inappropriate food intake, dietary intolerance
- Intussusception
- Parasitism
- Enteritis/colitis
- Septicaemia
- Toxaemia
- Yeast overgrowth/sterile gut syndrome following antibiotic therapy
- Inappropriate environment provision (e.g. lack of basking area, too cold, too hot, etc.)

FAILURE TO DEFECATE

- Inactivity
- Inappropriate temperature provision
- Intussusception
- Constipation
- Cloacolith/faecolith
- Follicular stasis
- Spinal neuropathy
- Metabolic disease (e.g. hypocalcaemia, dehydration, hepatopathy)
- See also Anorexia

SUBCUTANEOUS SWELLING

- Gout
- Infection
  - Abscess/fibriscess
  - Cellulitis
  - Granuloma
  - Mycobacteria

- Foreign body reaction
- Injection reaction
- Microchip
- Neoplasia
- Subcutaneous parasites (e.g. bot fly larvae)

GENERALISED OEDEMA

(real or apparent)

- Bladder rupture
- Excessive fluid therapy
- Hypoproteinaemia
    - Hepatopathy (hypoproteinaemia, effusion)
    - Nephropathy
    - Enteropathy
    - Starvation
- Congestive heart failure/circulatory failure
  The following will result in signs that mimic oedema
    - Coelomic infection/Yolk coelomitis
    - Prior freezing of limb extremities during hibernation
    - Toxins (e.g. stings, localised bacterial infection)
    - Gravidity
    - Dystocia
    - Cystic calculi
    - Coelomic granuloma
    - Follicular stasis
    - Obesity
    - Congestive heart failure
    - Intestinal disease/parasitism/obstruction
    - Coelomic infection/yolk coelomitis/gut perforation
    - Obstruction of the lower urinary tract
    - Hepatic lipidosis
    - Hepatic enlargement
    - Hexamitiasis/extraintestinal amoebiasis

COELOMIC SWELLING

(prefemoral distension)

- Gravidity
- Dystocia
- Cystic calculi
- Coelomic granuloma
- Follicular stasis
- Obesity
- Congestive heart failure
- Intestinal disease
- Coelomic infection, yolk coelomitis, gut perforation
- Hepatic lipidosis
- Hepatic enlargement
- Hexamitiasis, extra-intestinal amoebiasis

COELOMIC MASS

- Gravidity
- Follicular stasis
- Dystocia
- Urolith
- Intussusception
- Tumour
- Hepatic lipidosis
- Ectopic eggs

DYSTOCIA

- Inadequate nesting-site provision
- Competition for nesting sites
- Intra- or inter-species aggression
- Inability to exhibit nesting behaviour
- Inappropriate environmental provision (too hot, too cold, too dry, too humid, too dark, too bright, etc.)
- Maternal or egg morphologic and developmental abnormalities
- Mechanical obstruction
- Reproductive tract infections
- Systemic illness (hypocalcaemia, dehydration etc.)
- Endocrinopathy

PENILE PROLAPSE

- General debility
- Constipation
- Neurological dysfunction
- Excessive libido
- Mating injuries/trauma
    - Forced separation during copulation
    - Substrate contamination
    - Ground contact of engorged organ
    - Trauma during sex determination
- Hypocalcaemia and nutritional osteodystrophy may result in an inability to withdraw and retain the penis
- Cystic calculi or any other space-occupying coelomic lesion may result in tenesmus and secondary penile prolapse
- Bacterial, fungal, viral and parasitic infections of the lower genitourinary or digestive tracts

CLOACAL ORGAN PROLAPSE

- Depends upon the prolapsing structure, this should be identified wherever possible:
    - Bladder—e.g. urolithiasis
    - Shell gland—e.g. egg retention, salpingitis
    - Penis—e.g. trauma, infection
- Any coelomic space-occupying lesion or cause of straining (e.g. dyspnoea, constipation, egg retention, oviposition, etc.)
- Metabolic disease (e.g. dehydration, hypocalcaemia, ketoacidosis)
- Hyperoestrogenism and secondary cloacal hypertrophy (possibly)
- Obesity
- See also Penile Prolapse

CLOACAL HAEMORRHAGE

- Trauma (mating injuries, especially from male *Testudo hermanni*)
- Coagulopathies
- Cloacal infection
- Fly strike
- Parasitism

- Intestinal disease
- Dystocia

JOINT SWELLING

- Infection
- Trauma (e.g. fracture, dislocation, ligament rupture)
- Metabolic bone disease (*osteodystrophia fibrosa*)
- Gout
- Neoplasia
- Pseudogout
- Degenerative joint disease (osteophyte development)

LAMENESS

- Gout
- Fracture
- Ligament rupture
- Dislocation
- Neurological disease
- Metabolic disease
- Localised infection
- Neoplasia

TRAUMA

- Dog bites
- Rat bites
- Burns (e.g. wild animals in forest fires, inappropriate heat sources and confinement in terrestrial species)
- Hit by car
- Hit by lawnmower (terrestrial tortoises)
- Hit by speedboat (marine species)
- Pathological fractures (e.g. bone/joint collapse secondary to metabolic bone disease/hyperparathyroidism)
- Damage to nails through inappropriate substrate provision or excessively small enclosure in combination with persistent escape attempts or pacing
- Inappropriate confinement with other animals (intra- or inter-species aggression)
- Mating trauma (e.g. the mature male *Testudo hermanni* has a pointed claw on the end of its tail that can seriously tear the cloaca of any chelonians inappropriately confined with it)

EXCESSIVE ODOUR

- Cloacal infection/fly strike
- Plastronal infection/necrosis
- Dermal infection/necrosis (+/– septicaemia)
- Stomatitis
- Ketoacidosis (possibly)

DERMATITIS

(+/– septicaemia)

- Hypervitaminosis A
- Bacterial infection (e.g. subcutaneous abscess/fibriscessation or cellulitis)
- Viral infection (e.g. pox virus, iridovirus, herpesvirus)
- Fungal infection
- Burns
- Ectoparasites
- Chemical irritation (e.g. through excessive use of bleaches or other disinfectants)
- Inappropriate/excessive humidity
- Damage from abrasive enclosure substrate, poor enclosure construction
- Confinement of inactive species with inappropriately-managed heat sources (heat-mat blisters etc.)

EXCESSIVE SLOUGHING OF SKIN

(T-shirt and shorts) (+/– septicaemia)

- Generalised nutritional disease
- Inappropriate husbandry resulting in abnormal shedding
- Hypovitaminosis A
- Hypervitaminosis A (e.g. iatrogenic)
- Mycotic infection
- Bacterial infection
- Viral infection
- Self-trauma
- The skin areas of chelonians traditionally covered by T-shirt and shorts in man have increased frictional movements in comparison to limb extremities and therefore may exhibit abnormalities of skin shedding (increase and decrease)

EXCESSIVE SHEDDING OF SCUTES

(+/– septicaemia)

- Nutritional disease (e.g. iatrogenic hypervitaminosis A)
- Metabolic disease
- Excessive growth
- Subscute infection
- Vascular disturbance to scute/underlying bone area (e.g. from a prior crush injury or exposure to excessive basking heat source)
- Inappropriate humidity or temperature (e.g. semi-aquatic species deprived of bathing areas)

EXCESSIVE SKIN SHEDDING

(abnormal skin shedding, moist exudative dermatitis (+/– septicaemia))

- Nutritional disease (e.g. hypervitaminosis A)
- Infections (e.g. bacterial, fungal, viral)
- Burns
- Frost damage and secondary avascular necrosis
- Poor vivarium hygiene
- Inappropriate environment provision
- Myiasis

SHELL ULCERATION

(+/– septicaemia)

- Trauma
- Infection
- Heat/frost necrosis
- Bot fly strike
- Vascular disruption to dermal bone

SHELL FRACTURE

- Lawnmower injury
- Car trauma
- Dog bite trauma
- Speedboat trauma
- Falling injury
- Crush injury

SHELL DISTORTION

- Iatrogenic (e.g. enrofloxacin toxicity in hatchling/juvenile specimens)
- Heat trauma, premature closure of growth plate
- Congenital deformity (e.g. inappropriate incubation conditions)
- Metabolic bone disease
- Excessive juvenile growth/accelerated growth
- Secondary nutritional hyperparathyroidism

PYRAMIDING OF SHELL

- Metabolic bone disease
- Excessive juvenile growth/accelerated growth
- Secondary nutritional hyperparathyroidism
- Low humidity in hatchlings (possibly)

FLAT SHELL

- Metabolic bone disease, hyperparathyroidism
- Excessive juvenile growth/accelerated growth
- Trauma

SOFT SHELL

- Metabolic bone disease
- Excessive juvenile growth/accelerated growth
- Hyperparathyroidism
- Infection
- Heat trauma

SHELL DISCOLOURATION

- Septicaemia
- Trauma
- Excessive oil application

OVERGROWTH OF BEAK AND NAILS

- Nutritional osteodystrophy
- Protein excess
- Accelerated growth of juveniles
- Lack of exposure to abrasive/hard food or substrate

PLASTRONAL LESIONS

- Septicaemia
- Thermal trauma, poor vivarium hygiene, poor management
- Necrotising infections

PLASTRONAL DISCOLOURATION

(+/– septicaemia)

- Septicaemia
- Burns
- Osteomyelitis
- Trauma
- Frost damage
- Disruption to shell blood (e.g. trauma, vascular flukes or fluke eggs)
- Sitting on colourful food items (e.g. beetroot)

SWELLING OF LATERAL HEAD

(+/– septicaemia)

- Ear abscess
- Gout

BURNS

- Inappropriate heat source (too hot, wrong type, heating from below in basking species, etc.)
- Inappropriate confinement of inactive specimens beneath basking lamps or upon heat mats
- Heat trauma: forest fire (wild specimens), bonfire (captive specimens)

DEFLATED LIMBS

- Dehydration
- Cachexia/wasting
- Inactivity, lack of encouragement to exercise, muscle wastage, neuropathy, myopathy

SUNKEN EYES

- Dehydration
- Cachexia
- Any chronic disease
- Any acute disease

DECREASED SKIN ELASTICITY

- Dehydration
- Old age
- Cachexia/wasting

FAILURE TO URINATE

- Dehydration/prerenal azotaemia
- Urinary tract obstruction (e.g. renal gout, uroliths, coelomic mass)
- Physiological conservation of water during hibernation/aestivation
- Bacterial/viral or other nephritis
- Exposure to nephrotoxin (e.g. aminoglycoside therapy)

UROLITHS

- Dehydration and prolonged hibernation predispose to bladder urate deposits in uricotelic species. In addition to these expected urates, we have also seen apparent ammonium oxalate and cholesterol-containing calculi in urea-uricotelic species. Apatite (basic calcium phosphate), struvite (magnesium ammonium phosphate hexahydrate) and unidentified crystals have also been recorded. Nephrolithiasis has been reported in hypervitaminosis D.

URINE pH

- Acidic urine (<pH7) is suggestive of catabolism and is a consistent feature of herbivorous chelonians suffering from prolonged anorexia.

Fig. 11.1 *Testudo graeca* (*Testudo whitei*) (North African spur-thigh tortoise): The large lesion overlying the mandibular symphysis is probably an infected granuloma or fibriscess. Possible aetiological agents include bacteria, fungal agents and mycobacteria. Samples should be submitted for appropriate culture and sensitivity testing.

A formalinised sample should be submitted for histopathology. A non-formalinised sample should be stored for potential mycobacterial culture if histopathology or cytology suggests the presence of acid-fast organisms.

Fig. 11.2 *Testudo graeca* (*Testudo whitei*) (North African spur-thighed tortoise): Side view of the same animal as in Fig. 11.1.

Infections such as this might arise through foreign-body inoculation as a result of a shovelling action during feeding. This was a fungal infection.

LIMB TRAUMA

- Car trauma
- Child trauma
- Inappropriate handling by clinician and nursing staff
- Carnivore (dog, cat, racoon) bites
- Rat, mice bites
- Frost damage
- Inappropriate confinement
- Unsuitable substrate

GREEN URINE

(biliverdinuria)

- Suggestive of hepatopathy, cholestasis, bile-duct obstruction
- Haemolysis: chelonians lack the pigment bilirubin. The normal bile pigment (biliverdin) is green.
- Infectious agents/viral disease
- Hexamitiasis

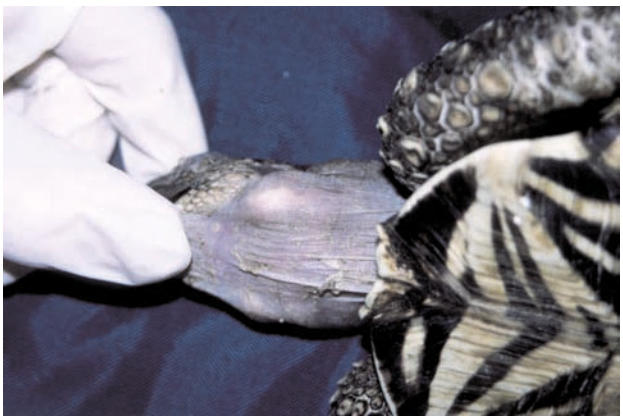

Fig. 11.3 *Geochelone elegans* (star tortoise): Enlargement of both temporomandibular joints in this individual was associated with a disseminated septic arthritis from which only fungal agents were identified. Other joints from this individual are illustrated later.

Fig. 11.4 *Testudo horsfieldi* (Afghan tortoise): This animal has shed the scales overlying its nasal chamber as a result of chronic upper respiratory tract disease. Animals such as this may be suffering from any combination of herpesvirus infection, mycoplasmosis or bacterial rhinitis. Most animals will recover and repair such lesions with adequate nursing and appropriate antibiosis.

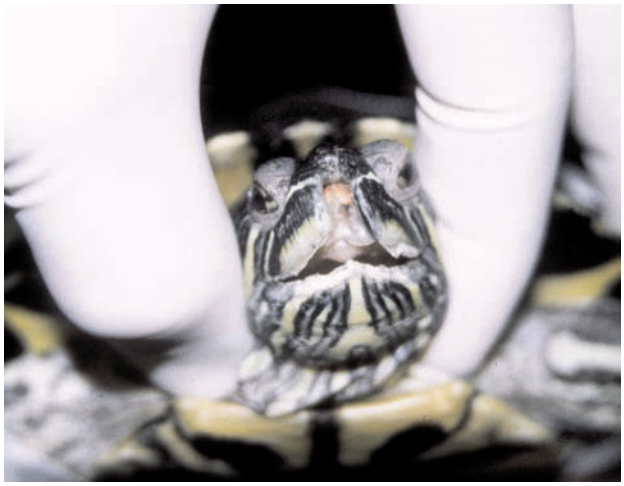

Fig. 11.5 *Trachemys scripta elegans* (red-eared slider): This animal appeared to have a congenital cleft-palate. It was untroubled by the absence of rhamphotheca, could eat normally and was presented for unrelated reasons.

Fig. 11.6 *Testudo graeca* (Moorish tortoise): Extensive loss of nasal structure as a result of a chronic mixed bacterial infection extending into both ears. This animal responded well to surgical debridement and antibiosis as illustrated elsewhere in this book.

Fig. 11.7 *Testudo ibera* (Turkish tortoise): Overgrowth of the rhamphotheca or upper beak is common. It may be associated with dietary excess of protein, deficiency of vitamin A or general neglect.

Fig. 11.8 *Testudo ibera* (Turkish tortoise): Side view of the same animal as in Fig. 11.7.

Fig. 11.9 *Testudo ibera* (Turkish tortoise): The same animal as Figs 11.7–11.8 following burring of the rhamphotheca. The keeper of this animal was unaware that the length of its beak was abnormal.

Fig. 11.10 *Geochelone sulcata* (African-spurred tortoise): Here an overgrown beak has resulted in a split of the rhamphotheca and horn covering the distal mandible. This in turn has produced impaction of food and infection at the base of the split. Sedation and resection of these lesions with a burr are required.

Fig. 11.11 The same animal as in Fig. 11.10 after sedation and burring.

Fig. 11.12 *Testudo ibera* (Turkish tortoise): Corneal opacity associated with reduced precorneal tear film and stomatitis consistent with herpesvirus infection.

Tear production increased and corneal opacity reduced as the stomatitis and the general demeanour of the animal improved with nursing and husbandry alterations.

Fig. 11.13 *Testudo hermanni* (Hermann's tortoise): *Keratoconjunctivitis* associated with herpesvirus infection and lymphoproliferative disease.

Fig. 11.14 *Testudo ibera* (Turkish tortoise): Lens opacity and haemorrhage into the anterior chamber of the eye hyphaema associated with freezing during hibernation.

The sight of these animals will improve over the following years with appropriate nursing and institutionalised or learned feeding regimes.

Fig. 11.15 *Geochelone pardalis* (leopard tortoise): Mild bilateral conjunctivitis from which *Mycoplasma agassizii* was isolated. This animal responded well to application of a topical tetracycline antibacterial eye ointment.

Fig. 11.16 *Testudo horsfieldi* (Afghan tortoise): Post-hibernation conjunctivitis in an animal from which both herpesvirus and *Mycoplasma agassizii* were isolated.

Occasional animals from a total group of 24 similar tortoises also had either stomatitis or rhinitis or combinations of all three signs.

The conjunctivitis was markedly pruritic. Blood is obvious on the forelimb as a result of continued self-trauma to the eyes.

Fig. 11.17 *Testudo ibera* (Turkish tortoise): Subpalpebral infection in a poorly-maintained post-hibernation animal. This animal damaged its eyes but the condition resolved with anti-mycoplasma antibiotic cover and improvements in husbandry (especially temperature) provision.

Fig. 11.18 *Chelonia mydas* (green turtle): Extensive proliferation of periocular fibropapillomatous material in a juvenile green turtle.

Fig. 11.19 *Trachemys scripta elegans* (red-eared slider): Conjunctivitis and caseous plaque typical of hypovitaminosis A.

Fig. 11.20 *Terrapene* (box turtle): The blepharospasm and conjunctivitis in this animal are non-specific but suggestive of hypovitaminosis A.

Fig. 11.21 *Testudo marginata* (marginated tortoise): This author (SM) has recorded a liver vitamin A value of 0.36 µg/g wet weight in a juvenile *Testudo marginata* with clinical signs consistent with hypovitaminosis A, including a caseous bilateral conjunctivitis, rhinitis, hepatitis and pneumonia (noted at post mortem examination).

Using a conversion factor of 3.36, this was equal to 1.2 IU/g.

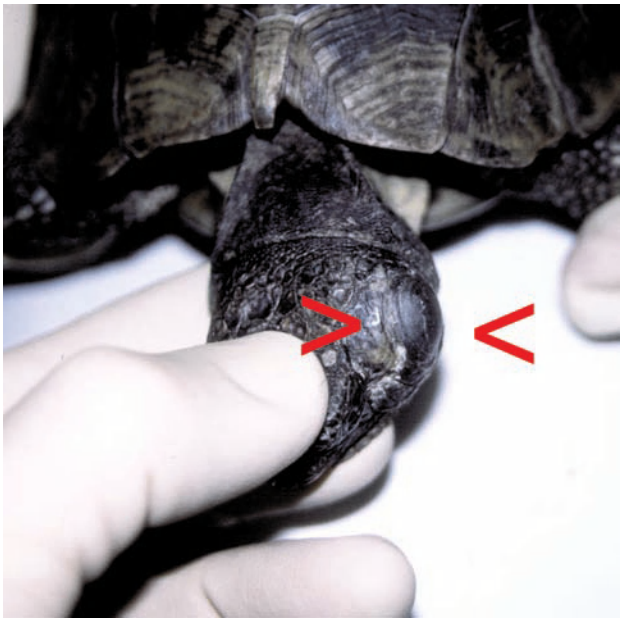

Fig. 11.22 *Testudo graeca graeca* (spur-thighed Moroccan or Moorish tortoise) (dorsal view): Lateral swelling of the head in the region of the tympanic scute (arrows). This is the result of a chronic ear abscess.

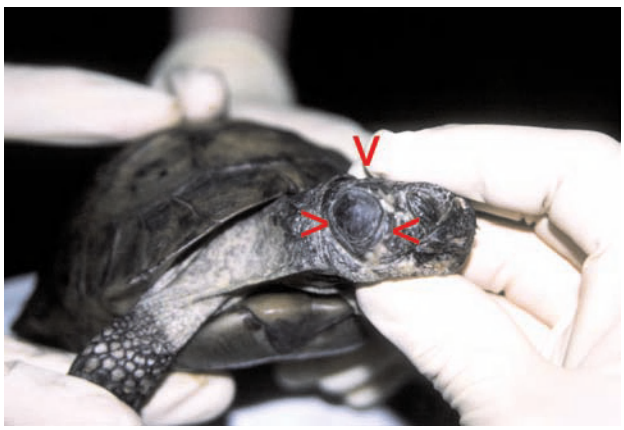

Fig. 11.23 *Testudo graeca graeca* (spur-thighed Moroccan or Moorish tortoise) (lateral view): Chronic ear abscess in same animal as Fig. 11.22.

Fig. 11.24 *Trachemys scripta elegans* (red-eared slider): Lateral view of an ear abscess (arrows).

Fig. 11.25 *Testudo hermanni* (Hermann's tortoise): Skin lesion in prefemoral fossa, above and relating to a plastron osteotomy site.

Infection, compromised blood supply and urine and faecal contamination of the site in immobile animals heated from below are all predisposing factors.

Fig. 11.26 *Testudo hermanni* (Hermann's tortoise): Infection of the skin of the ventral prefemoral fossa.

This animal was immobile and had been heated from below using a heat mat. Urine and faecal contamination of the site in combination with ideal conditions for bacterial incubation were causative factors.

The site was cleaned and ventilated by placing the animal on draining board matting. Heat was provided from above, and the lesion resolved with an antibacterial ointment.

Fig. 11.27 *Testudo graeca* (spur-thighed tortoise): Bacterial skin infection in an animal housed outside in the northern United Kingdom without supplementary heat or light provision (April).

The animal had a core body temperature below 15°C was leucopaenic and spent most of its time with its head retracted deep within its shell.

The site was cleaned and the lesion healed with systemic antibiotic therapy in combination with application of an antibacterial ointment and major improvements in husbandry.

Fig. 11.28 *Testudo hermanni* (Hermann's tortoise): Skin-fold infection in the foreleg of a poorly-maintained animal. Management and outcome were similar to Fig. 11.27.

Fig. 11.29 *Trionyx* spp. (soft-shelled turtle): Bacterial infection of the skin of the neck.

Lesions such as these are easily sampled for cytology and culture and sensitivity testing. Inappropriate substrate, comprised of course gravel, is likely to have been an initiating cause.

Soft-shelled turtles require fine silt if lesions are to be avoided. Poor water quality with high bacterial counts will be a compounding problem. Lesions resolved with improvements in husbandry and systemic antibiotics.

Fig. 11.30 *Trachemys scripta elegans* (red-eared slider): Limb lesion resulting from excessive rubbing against the side of its tank during attempts at escape. Water filtration was inadequate and bacterial challenge high.

Fig. 11.31 *Trachemys scripta elegans* (red-eared slider): The same animal as Fig. 11.30 (hind limb).

Fig. 11.32 *Trachemys scripta elegans* (red-eared slider): The same animal as Figs 11.30–11.31 showing bacterial infection of the shell.

Bacteria were also apparent in blood smears indicating the animal was septicaemic. This animal has septicaemic cutaneous ulcerative disease (SCUD).

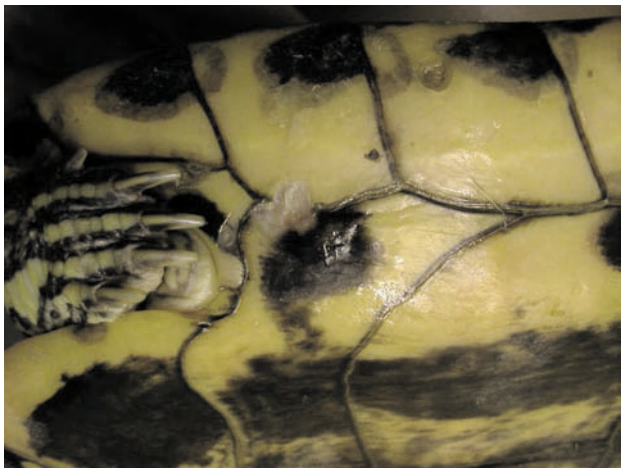

Fig. 11.33 *Trachemys scripta elegans* (red-eared slider): This animal was maintained in cold water without a haul-out area or water filtration. Water changes were infrequent.

Lesions such as this are easily sampled for cytology and culture and sensitivity testing. An area of scute is peeling away from the bridge of this animal. The area below is soft and has a pungent smell. Blood smears revealed bacteria. This animal has septicaemic cutaneous ulcerative disease (SCUD).

Fig. 11.34 *Testudo ibera* (Turkish tortoise): Dysecdysis, abnormal retention of partially shed skin, in a poorly-maintained tortoise living in a garden in the northern United Kingdom all year round without supplementary heat or light.

This animal benefited from improvements in husbandry and its skin looked normal after about a year.

Fig. 11.35 *Testudo hermanni* (Hermann's tortoise): Hypervitaminosis A.

This Hermann's tortoise was administered vitamin A by injection on four or more separate occasions by various veterinarians in a multivet practice, none of whom made a specific diagnosis following its presentation.

The soft skin of the proximal fore and hind limbs has sloughed and there is a moist, exudative dermatitis. This animal requires analgesia, antibiotics, fluids, extensive long term nursing and no more vitamin A. Recovery took around a year.

Fig. 11.36 *Geochelone sulcata* (African spurred tortoise): Hypervitaminosis A. This animal had a history similar to that in Fig. 11.35.

Fig. 11.37 *Trachemys scripta elegans* (red-eared slider): A red flush to skin strongly suggestive of septicaemia. Bacteria and toxic heterophils may be present in blood smears.

Fig. 11.38 *Testudo marginata* (Marginated tortoise): The limb extremities are grossly swollen as a result of freezing (prolonged sub-zero temperature exposure) during hibernation (frostbite). This animal was also blind and moving in circles.

The animal was provided with analgesia, fluids and appropriate nursing. The nails and some distal limb tissue required removal but the animal made a reasonable recovery and even regained some vision in the following years.

Fig. 11.40 *Testudo ibera* (Turkish tortoise): Peripheral oedema.

This animal was hyperuricaemic and in renal failure as a result of visceral/renal gout. Despite attempts to restore urine output, rehydrate the animal and achieve glomerular filtration, no urine was produced after two weeks' active fluid therapy.

Blood uric acid levels rose above 2000 μmol/l and blood potassium levels above 8.5 mmol/l. This animal was subsequently euthanased.

Fig. 11.41 *Geochelone elegans* (star tortoise): Granulomatous septic arthritis associated with the presence of an unidentified mycotic agent. The same animal as in Fig. 11.3.

Fig. 11.39 *Testudo graeca graeca* (spur-thighed Moroccan or Moorish tortoise): Gout. Abnormal stifle.

Radiography revealed extensive mineralised deposits within the joint capsules of many joints. This animal was hyperuricaemic with a blood level of 1860 μmol/l.

Normourica does not exclude the possibility of gout from previous episodes of hyperuricaemia.

Fig. 11.42 *Geochelone elegans* (star tortoise): Granulomatous septic arthritis associated with the presence of an unidentified mycotic agent. The same animal as in Figs 11.3 & 11.41.

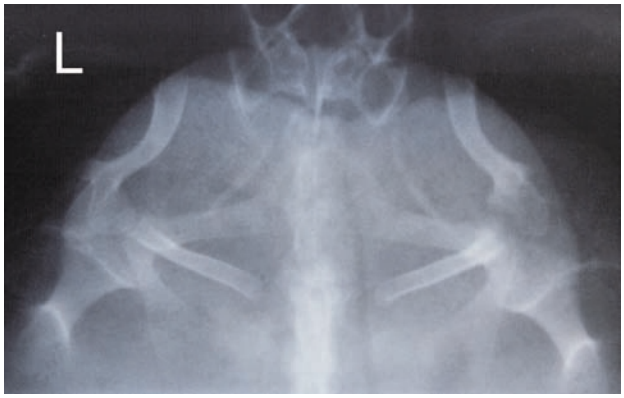

Fig. 11.43 *Pseudemys scripta elegans* (red-eared slider): Radiograph of a red-eared slider with septic arthritis of the right shoulder joint.

Chelonian septic arthritis typically exhibits lysis with little surrounding sclerosis.

Fig. 11.46 *Testudo ibera* (Turkish tortoise): Occasionally, when a rat gains access to the deeper tissues of the limb, fleshy tissues are gnawed away leaving an empty sleeve of skin.

In this case, the carapace also shows evidence of rat damage.

Fig. 11.44 *Testudo graeca graeca* (Moorish tortoise): A limb following recovery from earlier rat bite trauma during hibernation. Use of this stumpy limb was virtually normal.

Fig. 11.47 *Geochelone pardalis* (leopard tortoise): Accelerated growth and early maturity. A four-year-old captive bred and reared tortoise.

The animal is pictured alongside a 12-inch plastic ruler. See Fig. 11.48 for comparison.

Fig. 11.45 *Testudo ibera* (Turkish tortoise): Rat-bite trauma is a common presentation in post hibernation animals.

The exposed surfaces of the tail, hind limb and forelimbs are the usual sites affected. Occasionally eye and other head trauma may occur but this is unusual. Mild superficial injuries, such as this skin deficit in a forelimb, will heal quickly within a month or so.

The clinician should provide pain relief, antibiotic cover, wound dressings and effective environment and nutritional management.

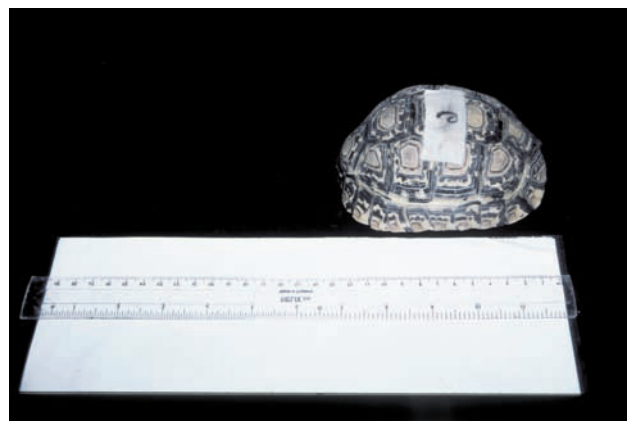

Fig. 11.48 *Geochelone pardalis* (leopard tortoise): Normal growth rate of a wild-caught, approximately four-year-old leopard tortoise.

The animal is pictured alongside a 12-inch plastic ruler. See Fig. 11.47 for comparison.

Fig. 11.49 *Testudo graeca* (North African spur-thighed tortoise): A year old hatchling with a hard, well-calcified shell that cannot be compressed by a human hand.

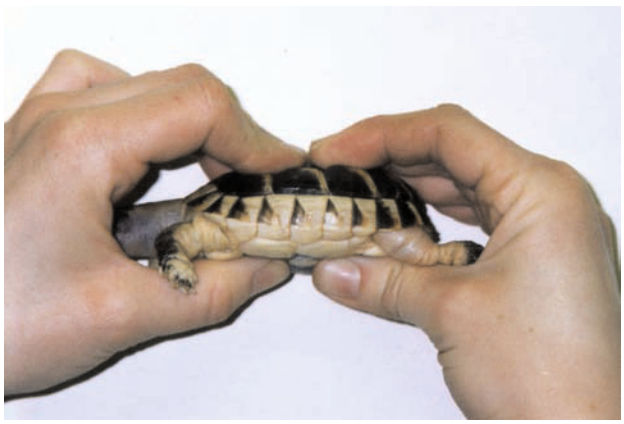

Fig. 11.50 *Testudo graeca* (North African spur-thighed tortoise): A year old hatchling with a soft, poorly-calcified shell that is easily compressed. (NB this animal is already dead following euthanasia.)

Compare this soft shell with the animal in Fig. 11.49.

Fig. 11.51 *Testudo hermanni* (Hermann's tortoise): Accelerated growth/soft shell in a two-year-old animal.

Inadequate calcification of the shell, combined with excessive weight, has resulted in deformity. The pull of the pectoral and pelvic muscle masses creates a carapace that rises from the nuchal scute to a point over the pectoral girdle. The carapace then slopes backwards caudally and may even become indented over the pelvic muscle-mass attachment points. The hind legs tend to splay outwards and point out behind the animal rather than below it to produce support. Such animals often drag their caudal plastron along the ground.

Fig. 11.52 *Testudo ibera* (Turkish tortoise): Pyramiding of the scutes of the carapace.

The increased rate of growth of the central region of each of the scutes of the dorsal carapace results in this knobbly appearance. Abnormal growth like this may be a combination of the effects of over-nutrition and inappropriate environment (e.g. too warm, too long a photoperiod or too low humidity in a basking vivarium with inadequate shelter provision).

Fig. 11.53 *Trachemys scripta elegans* (red-eared slider): Gross deformity of the carapace and plastron as a result of the effects of forces from the pectoral and pelvic muscle masses on a soft, poorly-calcified shell. The shell of this animal looks far too small for the body it contains.

Fig. 11.54 *Testudo ibera* (Turkish tortoise): Pyramiding of the scutes and a long, low, flat dome to the carapace (lateral view). Also see Fig. 5.52.

Fig. 11.55 *Testudo ibera* (Turkish tortoise): Accelerated growth and constriction of the cranial carapacial opening (cranial view). The same animal as Fig. 11.54.

Fig. 11.56 *Testudo ibera* (Turkish tortoise): Dorsal view. The same animal as Figs 11.54–11.55.

Fig. 11.57 *Trachemys scripta elegans* (red-eared slider): A combination of metabolic bone disease and inadequate water quality have led to a necrotic, infected plastron and carapace.

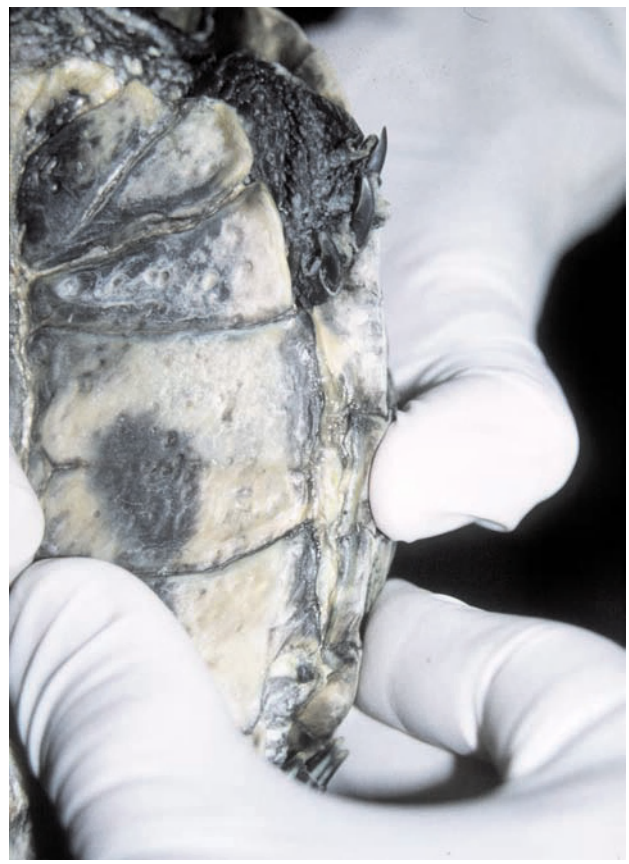

Fig. 11.58 *Trachemys scripta elegans* (red-eared slider): Close up of the plastron of the same animal as Fig. 11.57.

Fig. 11.59 *Trachemys scripta elegans* (red-eared slider): Metabolic bone disease during juvenile growth can result in facial deformity and the creation of spikes and spurs on the plastron or carapace.

Tension on the skeletal structures of the jaw and skull pull the face into abnormal, disfigured shapes.

Fig. 11.60 *Testudo hermanni* (Hermann's tortoise): Abnormal dorsal carapacial growth plate closure and increased secondary growth from the lateral marginal growth plates.

A tiny shell, derived from the hatchling carapace, sits on top of a large deformed mass of carapace and plastron.

Possible causes of this shell deformation include heat or frost trauma to the dorsal carapace. Alternatively it is possible that heat has only been provided ventrally from heat mats and the combination of ventral heat and humidity has resulted in disproportionate growth.

Fig. 11.61 *Testudo hermanni* (Hermann's tortoise): The problem illustrated in Fig. 11.60 was not restricted to a single animal.

Fig. 11.62 *Testudo hermanni* (Hermann's tortoise): Dorsal view of the same animal as Fig. 11.60.

Fig. 11.63 *Trachemys scripta elegans* (red-eared slider) (cranial view): Shell deformity.

The shell of this animal has been pulled in to conform to the contours of underlying musculoskeletal structures, suggesting delayed calcification during the juvenile/adolescent period.

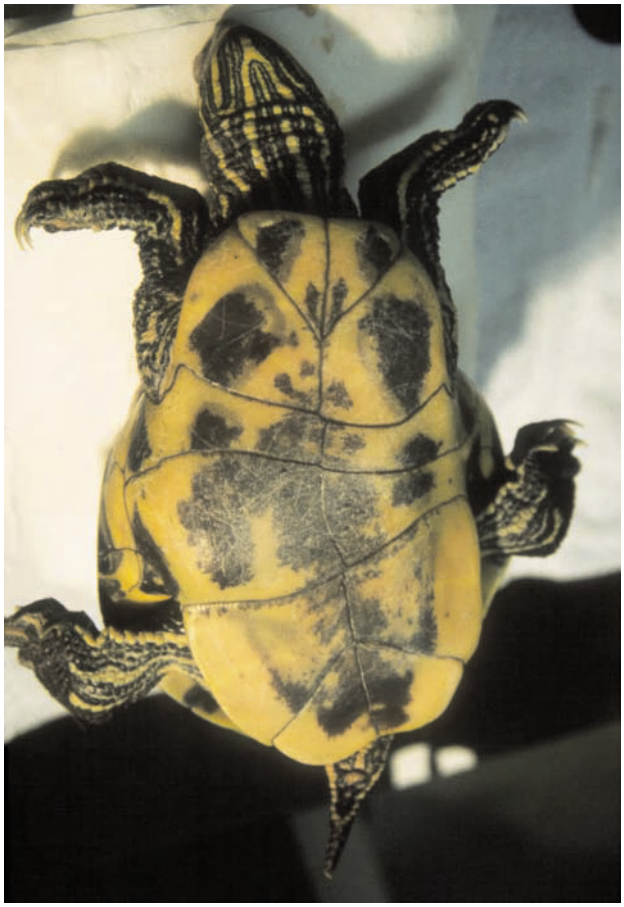

Fig. 11.64 *Trachemys scripta elegans* (red-eared slider (ventral view)): The same animal as Fig. 11.63.

Fig. 11.66 *Testudo hermanni* (Hermann's tortoise): A chronic example of shell damage such as that illustrated in Fig. 11.65.

Fig. 11.65 *Testudo hermanni* (Hermann's tortoise): Superficial shell trauma as a result of inappropriate confinement with an aggressive male.

Repeated butting has resulted in damage to superficial scute layers. This in turn can predispose to fungal and bacterial infections, which start to under-run neighbouring scutes producing a more generalised problem.

Fig. 11.67 *Trachemys scripta elegans* (red-eared slider): Poorly-calcified shell of an animal with nutritional secondary hyperparathyroidism and extensive secondary bacterial infection of the shell, resulting from inadequate water quality and lack of a haul-out area.

Fig. 11.68 *Geochelone sulcata* (African spurred tortoise): The result of ventral heat trauma.
A combination of excessive ventral heat from a non-thermostatically controlled heat mat, and the effects of urine and faeces, has resulted in an extensive infection which tracks out under the entire plastron. The scutes are easily lifted away from the plastron, the bone of which has a pungent-smelling necrotising infection.

A suitable treatment plan involves:

- microbial culture and sensitivity;
- appropriate systemic and topical antibiotics;
- removal of as much devitalised material as possible;
- topical barrier preparations (cream +/– dressings);
- raising the plastron to avoid ground contact. (Options include maintaining on a slatted tray or fixing support pegs to the periphery of the animal's plastron)

Fig. 11.69 Generalized *Aeromonas hydrophila* infection in an *Emydura subglobosa*. Dorsal view.

Several animals were kept in a small tank with no water filter, inadequate heat provision and no ultraviolet lighting. The water was only changed if and when it become turbid.
(Courtesy of Jean Meyer)

Fig. 11.70 Generalized *Aeromonas hydrophila* infection in an *Emydura subglobosa*. Ventral view.

Lesions arise at points of contact between the plastron and enclosure. The soft tissue in front of the hind limbs is also affected as a result of skin fold infection when the legs are retained in flexion.
(Courtesy of Jean Meyer)

Fig. 11.71 Generalized *Aeromonas hydrophila* infection in an *Emydura subglobosa*.

Here there is an obvious middle ear infection.

Bacteria are also likely to be seen on examination of blood smears, as such animals are generally bacteraemic. This can be categorised as SCUD.
(Courtesy of Jean Meyer)

Fig. 11.72 Generalized *Aeromonas hydrophila* infection in an *Emydura subglobosa*.

Lesions resolved with four weeks of antibiotic treatment as indicated by sensitivity testing, in combination with improvements in husbandry and nutrition.

An external water filter with activated charcoal substrate improved the water quality, and further improvements were made in light and temperature provision.
(Courtesy of Jean Meyer)

Fig. 11.73 Red-eared slider (*Trachemys scripta*). Lateral view.

This slider was presented because its carapace was disintegrating. The marginal scutes fell off and revealed the coelomic cavity. The underlying bones were spongy and soft. The animal was still alive on presentation.

It seems probable that this condition resulted from insufficient ossification due to lack of ultraviolet light exposure and an inappropriate raw meat diet without vitamin or mineral supplementation. It is also probable that a secondary bacterial infection of the coelomic cavity was present.
(Courtesy of Jean Meyer)

Fig. 11.74 The same animal as Fig. 11.73. Dorsal view.

The animal was euthanased and the owner requested no further investigation.
(Courtesy of Jean Meyer)

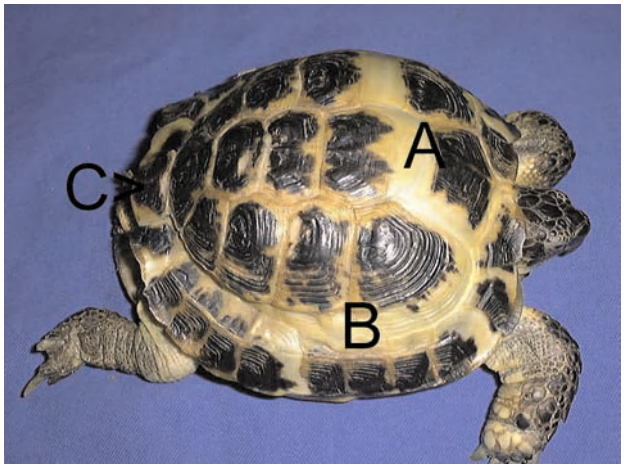

Fig. 11.75 Juvenile Afghan tortoise (*Testudo horsfieldi*). Accelerated growth and early maturity.

Abnormal growth pattern of the carapacial scutes, possibly due to inappropriate incubation management in combination with an excessively high juvenile growth rate.

(A) The anterior carapace has become raised. This shape conforms to the pectoral muscle mass and limb girdle and shape alteration is likely to be the result of muscular tension on a soft, rapidly developing carapace.

(B) There is evidence of significant growth from the lateral growth plates of the pleural scutes (yellow colour).

(C) The junction between the vertebral and marginal scutes is indented caudally as a result of muscular tensions from the muscles of the pelvic muscle masses.

(Courtesy of Jean Meyer)

Fig. 11.77 Radiograph of the animal in Fig. 11.76.

There is an absence of bony structure within the shell and deformities of the long bones of the limbs. Skeletal structures are ghost like.

This animal is likely to be suffering from hyperparathyroidism and may subsequently go into renal failure.

(Courtesy of Jean Meyer)

Fig. 11.76 Juvenile *Testudo marginata* with severe metabolic bone disease (MBD).

The shell was very soft and could easily be impressed.

(Courtesy of Jean Meyer)

Fig. 11.78 Distortion of the hind limbs due to lack of ossification of long bones which are soft and deform under the influence of the animal's weight (same animal as in Figs 11.76–11.77).

Such animals can be supported on wheels and similar structures until their bones are strong enough to support them. Such support encourages normal propulsive movements and retains muscle mass and tone.

(Courtesy of Jean Meyer)

Fig. 11.79 Red-eared slider (*Trachemys scripta*).
This animal was maintained outdoors in northern England without water filtration, supplementary heating, supplementary lighting, supplementary nutrition or supplementary UVB provision.
It was presented to the author with evidence of extensive advanced infection of the shell, skin, conjunctivae and mouth. It animal was making laboured respiratory movements.
Bacteria were apparent in blood smears and the case could be categorised as SCUD.

Fig. 11.81 The same animal as Fig. 11.79.

Fig. 11.82 *Testudo graeca* (Moorish tortoise): Bacterial infection of the skin of the tail and subcarapacial tissues.
This infection appeared to be the result of inappropriate temperature provision and care and resolved with improvements in husbandry, systemic antibiotics in combination with regular lesion care and topical antibacterial creams.

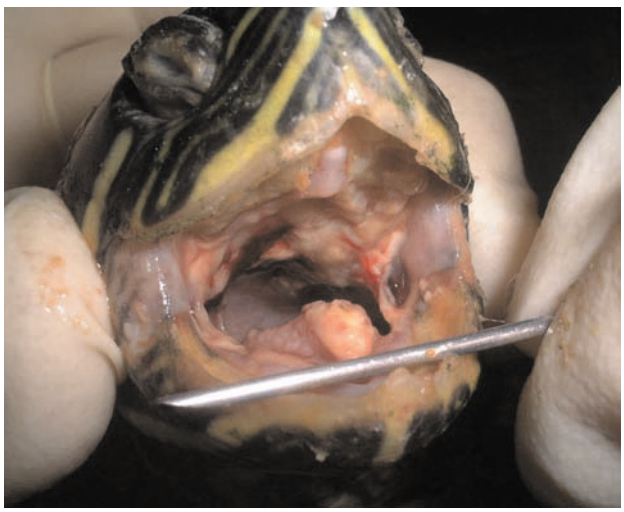

Fig. 11.80 Red-eared slider (*Trachemys scripta*).
Stomatitis can be the result of bacterial infection of poorly maintained animals provided with inappropriate nutrition, hygiene and temperatures.

Fig. 11.83 *Testudo hermanni* (Hermann's tortoise): Discharging fistula in the carapace of a mature female. This related to extensive underlying lung pathology.

Fig. 11.84 *Testudo ibera* (Turkish tortoise): Dry, ulcerative shell disease.
Here the scutes are easily lifted away from avascular bone below. This type of dermatopathy is generally progressive and is often associated with mycotic agents found in soil.

Fig. 11.85 *Testudo ibera* (Turkish tortoise): Dry, ulcerative shell disease.
Possible aetiologies include heat or frost damage, crush injuries or the effects of systemic metabolic derangements or bacterial endotoxins. Loss of blood supply to the shell will result in death and sloughing of overlying tissues.

Fig. 11.88 Colour of the plastron of a young spur-thighed tortoise (*Testudo graeca*) after it has walked through beetroot.
This pigment contamination is a benign differential to reddening of the plastron associated with septicaemia.
(Courtesy of Jean Meyer)

Fig. 11.86 *Testudo hermanni* (Hermann's tortoise): The red flush of this plastron suggests septicaemia.

Fig. 11.87 *Geochelone sulcata* (juvenile): The red flush of this plastron suggests septicaemia.

Fig. 11.89 *Terrapene ornata* (ornate box turtle) The carapacial lesions on this animal are consistent with previous fly strike injuries and secondary bacterial and/or mycotic infection.

Fig. 11.90 *Terrapene ornata* (ornate box turtle): The same animal as Fig. 11.75. Lesions on plastron.

Fig. 11.91 *Testudo graeca* (Moorish tortoise): Extension of the neck and open mouth breathing are consistent with lower respiratory tract disease and carry a guarded prognosis.

Fig. 11.92 *Testudo graeca* (Moorish tortoise): Pharyngeal swelling/oedema.

This may be due to goitre, virus-associated stomatitis, a consequence of carotid artery venepuncture, or part of more generalised oedema relating to fluid overload or hypoalbuminaemia (these may possibly be associated with hyperuricaemic renal failure).

Fig. 11.93 *Testudo graeca* (Moorish tortoise): Depression is a non-specific sign requiring comprehensive work up.

This animal could be dehydrated, too cold, septicaemic or suffering from viral associated disease. Many other differentials are also possible.

Fig. 11.94 *Testudo ibera* (Turkish tortoise): Depression is a non-specific sign requiring comprehensive work-up.

Fig. 11.95 *Geochelone pardalis* (leopard tortoise): Depression is a non-specific sign requiring comprehensive work up.

Fig. 11.96 *Testudo ibera* (Turkish tortoise): Presented by the client because it was inactive.

Twenty-six maggots were recovered from the mouth. The animal had been dead for some time.

Fig. 11.97 *Testudo graeca*: Normal presentation of penis, immediately prior to urination.

Fig. 11.98 *Geochelone elegans*: Moderate penile prolapse associated with a radio-opaque cloacal mass (cloacalith) similar to Fig. 11.85.

The prolapse itself is unlikely to require surgery, but the underlying cause must be determined and addressed.

Fig. 11.99 Cloacaliths like this may predispose to cloacal organ prolapse (see Fig. 11.84).

Survey radiography is indicated as part of the pre-surgical patient assessment protocol of any cloacal organ prolapse.

Fig. 11.100 *Testudo graeca*: Prolapse of hyperplastic cloaca in a female.

Fig. 11.103 *Testudo graeca* (*Testudo whitei*): On closer examination the central mass was identified as a peach stone.

Fig. 11.101 *Testudo hermanni*: Oviductal prolapse.

Fig. 11.104 Stance of a constipated Hermann's tortoise (*Testudo. hermanni*).
In this case straining as the result of a substrate-impacted colon.
(Courtesy of Jean Meyer)

Fig. 11.102 *Cuora* sp.: Necrotic penile prolapse.
This prolapse had been attacked and bitten by other animals in the same collection and amputation was necessary.

Fig. 11.105 Infection of preovulatory follicles can be associated with yolk coelomitis and septicaemia. Various bacteria were cultured directly from the yolk.
(Courtesy of Jean Meyer)

PROBLEM-SOLVING APPROACH TO CONDITIONS OF MARINE TURTLES

STUART MCARTHUR

The following section is intended as a quick and easy guide for clinician use at the animal's side. Where conditions are complex or multifactorial the index can be used to cross-reference to other sections, and where conditions are dealt with in greater depth elsewhere in the book the reader is also directed to these other locations.

HYPOGLYCAEMIA

Aetiology

- Starvation, anorexia, debility.
- Potentially a problem for all wild-captured chelonians and many ill aquarium-managed turtles.

Clinical signs

- Animals are weak and depressed.
- Chronic disease may also manifest itself in wasting.

History

- Non-specific.
- Potential to occur in any starved animal.
- Wild animals may be cold stunned, injured, have suffered entrapment or boat trauma or may be suffering with chronic infectious disease (e.g. CNS/vascular trematodes, fibropapillomatosis).

Diagnosis

- Relatively easily measured at the animal's side during initial evaluation as covered earlier in this book. Blood glucose sticks intended for human use are suitable.
- Normal glucose measurements are poorly defined and will vary with sex, age, species and reproductive activity.
- Normal values (season not specified) are given as 3.3–6.7 mmol/l (60–120 mg/dl) (Campbell 1996b). Walsh (1999) gives values slightly higher.

Treatment

Oral, intravenous and intracoelomic supplementation. Response to treatment should be carefully assessed using regular blood glucose measurements.

Oral supplementation
- Oesophageal and orogastric intubation.
- Useful for ongoing management after initial intravenous or intracoelomic stabilisation.
- Suitable for relatively stable animals with functional digestive tracts.
- Relatively slow rates of absorption in debilitated animals.
- Complications such as aspiration of regurgitated fluid possible.
- Fluids given (e.g. 1 ml of 50% dextrose/kg 3–6 times daily) are relatively hypertonic and may complicate dehydration.

Intravenous and intraosseous supplementation
- Complicated.
- Catheters are poorly tolerated and hard to place.
- Constant monitoring essential.
- Useful if response to intracoelomic fluids is poor.
- A bolus IV dose can be administered to collapsed animals.

Intracoelomic supplementation
- 5% dextrose can be given at 11–17 ml/kg (Walsh 1999).
- Walsh (1999) describes a technique where the turtle is placed in dorsal recumbency with its caudal aspect tipped upwards to clear away viscera from the prefemoral injection site.

COLD STUNNING

Aetiology

- Often occurs where water temperatures fall below seasonal normal for the area.
- Juveniles of small body size have a high surface area to volume ratio and so chill quickly, rendering them more likely to develop problems than larger turtles.
- Turtles basking at the surface suddenly carried into cooler areas by alterations in currents. Such animals tend to float and have low levels of activity until either they are carried into warmer water or are rescued.
- Hypothermic animals become inactive and are prone to predation. Immunocompromise renders them prone to pneumonia and other infections.
- Tropical turtles believed to have been cold stunned can be carried vast distances in currents like the Gulf Stream. Possible cases have been washed up on United Kingdom coasts.

Clinical signs

- Animals are weak, cold, tend to spend much time floating at the surface and become uncoordinated and emaciated with time.
- Can appear similar to disease syndromes such as lethargic loggerhead syndrome, as animals will just be presented as cold and debilitated. Cold-stunned animals will have good corneal blink reflexes compared with diseased animals such as lethargic loggerheads.

- Where the animal is in good body condition it is likely that the thermal insult is recent. Such animals will often be well coordinated and able to raise their heads unaided.
- Monitor core body temperature where possible (e.g. Vet-Ox®, Heska).

History

- Larger turtles are less likely to be affected because of thermal inertia.
- Unlikely in leatherback turtles (*Dermochelys coriacea*), which are believed to have some ability to thermoregulate in colder seas.
- Juveniles are predisposed, especially basking species such as *Chelonia mydas*.
- May occur individually or in groups.
- Turtles found around coasts in latitudes outside the normal for the species should be considered cold stunned as a consequence of other trauma or disease.
- Trauma, temporary entanglement, hypoglycaemia and dysphagia following ingestion of plastic bags should be considered potential concurrent diagnoses.

Diagnosis

- Physical examination, blood profile and radiography serve as a basic assessment.
- Most animals will require assessment and therapy for hypoglycaemia.
- Consider investigating all suspected cases for more serious disease.
- Unresponsive cases and those with complications may need additional investigations.

Treatment

- Return core body temperature to normal range and provide nutritional and other appropriate support with a view to prompt release. Monitor core body temperature during recovery.
- Severely-chilled animals may require endotracheal intubation and resuscitation as described at the end of the Anaesthesia section in this book.

Mild cold stunning
- Place in warm water at 26°C.
- Monitor and release as soon as animal appears stable.

Moderate to severe cold stunning
- Basking lamps.
- Not appropriate to most marine species unless no other facilities to warm the animal are available. Skin and carapace should be protected from desiccation using barrier creams.
- Heat pads.
- Care should be taken to prevent localised thermal injury.
- The skin and carapace may benefit from protective creams, such as petroleum jelly, to reduce desiccation.
- Warmed fluids.
- Intracoelomic (IC) fluids are relatively straightforward to administer. Care should be taken to ensure thermal injury to viscera is avoided. Over-hydration may affect respiration and buoyancy. IC fluids should be combined with exogenous heat provision, for example with warm bathing and heat pads.
- Colonic/cloacal enemas are relatively difficult unless the patient is severely affected, in which case initial stabilisation may involve warmed cloacal washes.
- Intravenous fluids: Access is hard to maintain without complex catheterisation. The jugular or dorsal cervical sinuses are the usual sites. Cut-down may be necessary in severely dehydrated animals or those with reduced peripheral perfusion. Initial bolus therapy with warmed fluids containing glucose can be helpful.
- Intraosseous fluids: A needle with stylet is inserted into the distal quarter of the humerus at an angle of 30°–45° from parallel (Whitaker & Krum 1999). The catheterised limb can be folded and then taped in flexion within the carapace.

Severe cold stunning with respiratory compromise
- Resuscitate (techniques such as intubation and ventilation).
- Therapy to raise core temperature.

MORIBUND ANIMALS – RESUSCITATION

Aetiology

Hypothermia, anaesthesia or entrapment and anoxic submergence.

Clinical signs

- Turtles have become comatose and may appear dead or near death.
- Other disease may be present and may be a contributing factor.
- Concurrent anoxic acidosis may be present.

History

May have suffered forced submergence or entrapment within a captive enclosure.

Diagnosis

- Physical examination, evidence of corneal reflex, pain responses or cardiac output. Ultrasonography or Doppler cardiac assessment where possible.

Treatment

Several techniques have been used for the resuscitation of comatose sea turtles. Techniques described include electrical stimulation of pectoral musculature (Shoop 1982) and regular compression of the plastron whilst the turtle is in dorsal recumbency (Hopkins & Richardson 1984). However, Stabenau (1993) advises that these are both relatively ineffective. Balazs (1986) suggests intubation of the trachea with plastic tubing and ventilation and Stabenau (1993) suggests a modification of this technique using PVC pipe as a gag, through which a cuffed endotracheal tube can then be passed and the cuff inflated. Ventilating at 6–10 breaths per minute, using a hand held resuscitator bag, and increasing core temperature to the range 25°C–30°C have been

used by Stabenau to resuscitate a Kemp's Ridley turtle suffering from a severe anoxic acidosis following near drowning. Mechanical ventilation was required for 48 hours.

ENTANGLEMENT

Aetiology

- Marine turtles often become entangled in float lines for lobster pots etc. and long lines as opposed to nets. Potentially turtles may be attracted to buoys mistaking them for food.
- In many areas that are highly populated with turtles, compulsory turtle eliminating devices (TEDs) reduce the likelihood of nets catching turtles.

Clinical signs

- Injuries include vascular compromise of limbs, line-incision injuries to limbs and enforced submergence and drowning.
- Animals may be weak, cold and near drowning.

History

- Animals may be found entangled.
- Animals may have become cold stunned and be found drifting and weak.
- Flipper and other injuries may be apparent.

Diagnosis

- May be self-evident.
- Chronic head, limb and body entanglement injuries may be apparent.
- Otherwise, if found free-floating, will be categorised as, and therefore should be treated as, cold stunned and/or hypoglycaemic.

Treatment

Animals are likely to be exhausted, chilled and hypoglycaemic. Initial stabilisation as for these conditions.

Strong animals—mild injuries
Assess stronger animals with minor trauma or weakness with a view to releasing as soon as possible. Unnecessary capture, transportation and management of such animals may compromise survival rates.

Apparent drowning
Worth attempting intubation and resuscitation with animals suspected of forced submergence and drowning, as vascular shutdown and dive reflexes may facilitate recovery in a small percentage of such cases (see Moribund animals—resuscitation).

Complex injuries
More complicated injures to limbs and carapace may require surgical management as described elsewhere in this book (see Surgery and Boat Trauma).

GASTROINTESTINAL TRACT OBSTRUCTION

Aetiology

Species used to feeding on jellyfish will readily ingest floating plastic bags. These become impaled on the oesophageal digestive papilla as water is ejected orally during the swallowing process leaving partial or even complete oesophageal obstruction. Also often occurs where household and ship refuse is dumped close to turtle feeding grounds.

Clinical signs

- Any animal rescued and presented cold stunned should be considered a likely candidate for concurrent intestinal obstruction.
- Most damage from hooks will occur in the upper digestive tract.

Diagnosis

- Radiography will help reveal metal fishhooks.
- Oral endoscopy, though complex, will identify oesophageal plastic bags.
- Radiograph series using contrast media may be required to identify fishing line and rope which cause intestinal blockage and perforation.
- A common finding in post-mortem examination of dead strandings.

Treatment

- Endoscopy-assisted removal or oesophageal/coelomic surgery.
- Oesophageal obstruction may be relieved by hand in anaesthetised, gagged patients.
- Prevention: Complex—biodegradable bags and fishing line may help.

PARASITISM

Aetiology

- Young green turtles (*Chelonia mydas*) may have large burdens of tissue trematodes.
- Loggerhead turtles (*Caretta caretta*) may have nematodes and trematodes.

Clinical signs

Non-specific. Many centres in North America will treat all animals routinely upon capture from the wild. Marine turtles are commonly affected with Spirorchids (digenetic trematodes) and may suffer serious vascular disease or pneumonia (Wolke *et al.* 1982).

History

Non-specific. All rescued wild chelonians should be assumed to carry parasites.

Diagnosis

- Physical examination and a general health profile and radiography as a basic assessment.
- Faecal examination, colonic washes, post mortem findings.
- Distinctive orange or deep-yellow, thin-shelled, often operculated eggs in faeces. May contain discernible miracidia.
- Spirorchid eggs from sea turtles are elongate and have hooked terminal processes (George 1997).

Treatment

Use standard glucose and fluid administration protocols (see Hypoglycaemia), and the antiparasitic protocol set out below, during initial capture of all debilitated marine chelonians.

Antiparasitic protocol

- Nematodes: Fenbendazole at 50–100 mg/kg; repeat in 2 weeks
- Trematodes: Praziquantel 16 mg/kg; repeat in 2 weeks (Walsh 1999)

In loggerhead turtles, orally-administered praziquantel should be given three times at 25 mg/kg with three hours between doses (Jacobson *et al.* 2002).

Screen chronically-ill captive animals. Where possible remove the possibility of encountering intermediate hosts of parasites with an indirect life cycle.

FLOTATION ABNORMALITIES

Aetiology

The most common cause is escape of air from the respiratory tract secondary to infection or other pathology such as trauma. Increased buoyancy may also be associated with:

- generalised weakness and emaciation (e.g. following cold stunning or injury from a boat strike);
- gas-producing organisms in infected coelomic viscera or within the digestive tract;
- gas accumulation within the digestive tract following foreign-body obstruction.

Clinical signs

- Increased and abnormal flotation

Diagnosis

- Physical examination, blood biochemistry and haematology, and radiography as a basic assessment.
- Cases may need additional investigatory techniques, including respiratory endoscopy, examination of lung-wash material, biopsy of lesions and cytology, histopathology and appropriate viral/microbiological culture of material harvested from lesions. Many cases are treated by aspiration of coelomic air, however if there is an underlying cause this should also be investigated and managed appropriately.
- Work is currently under way to determine a reference database of normal coelomic pressure of marine turtles, in order to assist with removal of excessive gas from the coelomic cavity indicated through coelomic or whole-body radiography (Grimm 2001).

Treatment

- Use standard glucose/fluid/antiparasitic protocols (see Hypoglycaemia and Parasitism) during initial capture.
- Lowering salinity will aid turtles with flotation abnormalities e.g. due to excessive coelomic air, but normal turtles similarly housed will find they must work harder to maintain buoyancy.
- Aspiration of coelomic air during recovery from respiratory lesions. This may need to be done repeatedly until the primary cause of any respiratory pathology or gas production is addressed.
- It is hoped that the volume of gas requiring removal can be more acutely assessed once a database of normal coelomic pressures of various species at different ages is available (Grimm 2001).

FIBROPAPILLOMATOSIS

Aetiology

- The aetiology is unknown, but a herpesvirus is suspected. Transmission studies have demonstrated the presence of a transmissible agent. A potential cofactor is increased water temperature; retroviral agents and teratogens are also possible causes.
- High morbidity rates are experienced by populations around Hawaii and the southwest coast of the United States. Initially green turtles (*Chelonia mydas*) were considered the only affected species but more recently the disease has been described in several other species.

Clinical signs

- Multiple papillomatous growths around the eyes, cornea and skin of the head, neck, limbs, shell and tail.
- Lesions can be small (a few millimetres diameter) to large pedunculated masses of 20 cm or more.
- Lesions obstruct vision, compromise locomotion, increase predation and reduce the animal's ability to feed, resulting in emaciation.

History

- Prevalent in certain equatorial waters.
- Lesions are pathognomonic.

Diagnosis

Radiographic evaluation is helpful, as extensive and potentially necrotic visceral lesions carry a poor prognosis and may necessitate euthanasia.

Treatment

- Surgical removal of fibropapillomas affecting the well-being of individual turtles may be indicated, providing extensive

internal tumours are not apparent on radiographic or endoscopic examination (Schaff 1999: personal communication).
- General anaesthesia and/or analgesia are indicated.
- Lesions can be debulked or removed surgically under general anaesthesia once weak animals have been appropriately stabilised.
- Release should involve appropriate risk assessment, as it may involve introducing a known source of infection into wild populations.
- Long-term implications for affected populations are alarming, especially if the disease is partially the result of global warming and pollution.
- Regression of lesions as a result of host immune response and resistant individuals may see the stabilisation of what is becoming a multi-species pandemic.

PETROL AND OIL TOXICITY

Aetiology

- Both surface contamination and ingestion of toxic oil products.
- Follows illegal purging of fuel/oil tanks, spills and tanker groundings.

Clinical signs

Non-specific. Oil contamination may be obvious.

History

A recent local oil spill is possible. Other species may be similarly affected.

Diagnosis

- Usually self-evident. History and condition of other local animals helpful.
- May be a post-mortem finding.

Treatment

- External contamination can be removed using detergent baths, as with birds.
- Orally ingested oils can be broken down using oral fatty foods such as mayonnaise and carbon-based products, which bind oil products decreasing absorption and facilitating excretion.
- A dose of 2–8 g/kg/day of activated charcoal is suggested by Campbell (1996b).

TRAUMA

Aetiology

- Basking juveniles (e.g. *Chelonia mydas*) are predisposed to boat strikes as they feed in shallow water relatively close to coasts.
- Damage may be caused by propeller or hull impact.
- Fishermen may deliberately cause head trauma to turtles presumed to have reduced catches or damaged equipment.
- More disseminated trauma may result from dropping captured turtles on the deck of a boat.
- Other causes of trauma include shark-bite wounds, turtle-bite wounds (if stocking densities are inappropriate) and plastic carrier-rings from multi-pack beer cans encircling appendages and causing injury.

Clinical signs

- Boat strikes commonly result in head and carapace injuries.
- Occasional further trauma to shoulders, limbs, etc.
- Head trauma may affect salt-gland function.
- Disorientated animals are prone to secondary predation.
- Spinal trauma may result from carapacial strikes.

Prevention (Inshore management)

- Where possible, eliminate speedboats from areas frequently populated by turtles and restrict boats to a safe distance from shore.
- Boats should be slow and have an observer on the bow. Turtles sometimes appear suddenly in front of a boat, so controlling speed and watching are both important.

Diagnosis

- Lesions generally self-evident.
- Physical examination and a general health profile and radiography as a basic assessment.

Treatment

General

Use standard glucose/fluid/antiparasitic protocols (see Hypoglycaemia and Parasitism) at initial capture.

Superficial injuries

- Superficial injuries are often best left to heal by themselves; temporary confinement and protective antibiotic cover are sometimes beneficial.
- Where foreign debris is present or injuries appear chronic, wounds should be flushed with appropriate saline or dilute povidone-iodine solution.

Complex trauma

- Where there is more extensive trauma, such as skull fractures, stabilisation of bony fractures, supportive therapy and wound management are important.
- Chronic injuries may partially heal leaving fistula tracts to deeper pockets of infection and necrosis.
- Admit into a specialised hospitalisation facility as described earlier, or use flotation tanks and bathroom hospitalisation where no such facility exists.
- General anaesthesia and debridement and surgical removal of foreign/necrotic material and bony sequestra are likely to be required.
- Antibiotics such as enrofloxacin or ceftazidime may be helpful by injection (see Therapeutics).

Spinal injuries
Animals with spinal injuries will need appropriate viability and rehabilitation assessment.

Surgery
- Limb amputation is relatively simple and often well tolerated.
- Stabilisation of carapace and skull fractures is more specialised and where necessary carry a relatively guarded prognosis (see Surgery).

See advice on management of carapacial and head trauma in the Surgery section of this book.

CONSTIPATION

Aetiology

- Associated with emaciation, dehydration and debility.
- Common in animals feeding on animals with shells, where calcium-rich debris accumulates in the colon.

Clinical signs

- Non-specific.
- Weight loss, anorexia, lethargy.
- May follow any debilitating illness.

History

Absence of faeces.

Diagnosis

Radiography, palpation, endoscopy.

Treatment

- Surgery carries a poor prognosis as animals are often weak and anaemic.
- Medical therapy includes the use of intestinal stimulants and mineral oil.

Metoclopramide
- 0.5 mg/kg orally every 48 hours.
- 0.3 mg/kg daily by injection.

Mineral oil
- Juveniles 2.2–3 ml/kg; possibly mixed in gelatine.
- Adults 45 kg or more 1 ml/kg.

Walsh (1999) advises the return of turtles to water following oral administration of oil in order to reduce the risk of aspiration. Observe for defecation.

NUTRITIONAL PROBLEMS

Aetiology

- Where turtles are head started or wild hatchlings are taken illegally and raised in home aquaria on unsuitable diets.
- Diets for captive sea turtles restricted to freeze-dried krill, or squid and fish have been implicated as a cause of nutritional disorders (George 1997).
- George (1997) describes captive juvenile loggerhead turtles (*Caretta caretta*) fed a diet with Ca:P ratio around 1:1 as developing hyperparathyroidism, demineralisation of long bones and pathological bone fractures. He explains that the optimum dietary ratio of Ca:P in sea turtles has yet to be determined and describes a gelatine-based diet formulated to correct an unsuitable dietary ratio (Chromanski *et al.* 1987).

Treatment

- George (1997) describes the successful rearing of captive farmed turtles using commercial pelleted food, modified pelleted trout ration and gelatine diets.
- Commercial diets generally ranged from 25%–35% protein, but a 45% protein diet has been used with success in Kemp's Ridley turtles (*Lepidochelys kempii*).
- Metabolic bone disease may require dietary calcium, phosphorous and vitamin D assessment. Ensure that suitable access to sunlight and/or full-spectrum lighting is provided.
- Thiamine can be given to individuals maintained predominantly on fish. Dosing appears empirical.
- The requirements of hatchling green turtles (*Chelonia mydas*) for seven amino acids have been determined, and a requirement for vitamin A suggested (Bjorndal 1997).
- Severely anaemic animals may benefit from a single dose of vitamin K at 0.5 mg/kg (Walsh 1999).
- Iron toxicity is possible with supplementation but 0.5 mg/kg in divided doses over two weeks did not cause problems according to Walsh (1999).

EIMERIA AND CARYOSPORA

Clinical significance

- *Caryospora* and *Eimeria* in marine turtles.
- Over 30 species of coccidian are known in tortoises and turtles (McAlister & Upton 1989). Terrestrial chelonians infected with *Eimeria* are generally asymptomatic, although it has been suggested that infection may contribute to debility in sick animals (Barnard 1986a; Jacobson *et al.* 1994).

Eimeria caretta has been identified during routine faecal examination of loggerhead turtles, but the agent was not associated with intestinal disease (Upton *et al.* 1990; Jacobson *et al.* 1994).

Fatal ulcerative enteritis has been associated with *Caryospora cheloniae* identified in both wild (Gordon *et al.* 1993) and captive (Leibowitz *et al.* 1978) green turtles (*Chelonia mydas*). Clinical signs, histopathology and microbiological findings were similar in both groups, with large numbers of elongated oocysts seen. Pathological alterations were most pronounced in the caudal third of the large intestine where the lumen was grossly dilated and filled with blood, oocysts and tissue debris.

Diagnosis

(see also chapter on Clinical Pathology)

- Ovoid sporulated oocysts of *Eimeria* are typically 10–15 µm × 25–37 µm and found in faeces. Each contains four spherical sporocysts (Barnard 1986).
- *Caryospora* oocysts in marine turtles are elongate and described extensively by Leibowitz *et al.* (1978).
- Histopathology of tissues may be required to identify intranuclear coccidiosis, as faecal shedding is not always apparent.
- Jacobson *et al.* (1994) were unable to demonstrate the presence of faecal oocysts in infected *G. radiata*. Transmission electron microscopy was used to identify trophozoites, merozoites, microgametes and macrogametes.

Treatment

(see also chapter on Therapeutics)

Attempts to treat these animals have generally been unrewarding, although anecdotal reports indicate that toltrazuril (Baycox®) may be effective for this syndrome.

Sulpha drugs are potentially effective against *Eimeria*. Wallach & Boever (1984) suggest 1 oz of sodium sulfamethazine per gallon of drinking water for ten days. Trimethoprim/sulphadiazine has been reported at 30 mg/kg IM once daily for two days and 15 mg/kg IM every second day (Lane & Mader 1996). Klingenberg (1993) recommends using sulfadimethoxine at 50 mg/kg daily for three days, then repeating the course three days later.

Caryospora is reported to be unresponsive to sulphonamide therapy (George 1997). Hygiene is therefore important in control.

13 PROBLEM-SOLVING APPROACH TO COMMON DISEASES OF TERRESTRIAL AND SEMI-AQUATIC CHELONIANS

STUART MCARTHUR

The following section is intended as a quick and easy guide for clinician use at the animal's side. Where conditions are complex or multifactorial, the index can be used to cross-reference to other sections, and where conditions are dealt with in greater depth elsewhere in the book the reader is directed to these other locations.

ANOREXIA

Aetiology

Anorexia is a sign associated with a large number of chelonian husbandry problems, and can be associated with almost any acute or chronic disease.

The term *post-hibernation anorexia* is, however, misleading: in temperate latitudes the whole summer is 'post-hibernation', and anorexia is a sign associated with a large number of different situations throughout the year. The term is frequently used when in fact the condition results from a long-term failure to notice that a chelonian has been ill.

Post-hibernation anorexia may relate to:

- failure to observe that a confined animal is no longer in hibernation;
- failure to hydrate the animal suitably before and after hibernation;
- failure to feed the animal suitably before and after hibernation;
- failure to provide suitable heat and light before and after hibernation;
- an excessively long period of hibernation;
- disease or trauma acquired during the hibernation period (e.g. frost damage or rat bites);
- the culmination of chronic, undetected disease only noticed in the post-hibernation period.

A specific protocol for the management of post-hibernation hyperuricaemia, hyperkalaemia and anuria, which is one cause of post-hibernation anorexia, is given later. Complications associated with inappropriate hibernation are also described elsewhere.

Examples of common conditions associated with anorexia include:

- gastrointestinal disease (foreign body/impaction/parasitism);
- respiratory disease;
- nutritional disease;
- dehydration;
- ketoacidosis/azotaemia/hypocalcaemia/hyperkalaemia/hypokalaemia;
- other electrolyte imbalances;
- renal failure;
- debility associated with inappropriate or excessive hibernation;
- follicular stasis;
- egg retention;
- hepatic disease (lipidosis/hepatitis);
- sight impairment (frost damage, intraocular disease);
- central nervous system disease;
- inappropriate environmental provision (too cold, too dark, too humid, too dry, too hot etc.);
- inappropriate food provision (meat to a herbivore, lettuce to an omnivore etc.);
- pain (e.g. undiagnosed injury);
- maladaptation (e.g. sudden environment change in captivity following wild capture);
- social disruption (e.g. loss of vivarium mate/intra/inter species aggression);
- psychological (e.g. post dog bite/transportation stress);
- advanced folliculogenesis in breedable/reproductively active females (normal);
- aestivation in normal animals in good weather (especially *Testudo horsfieldi*) (normal);
- behavioural (e.g. male intent on mating/female during ovulation /oviposition) (normal);

Clinical signs

Further signs accompanying anorexia vary depending with the cause of the problem.

History

Varied. Details from the history will give a large number of clues as to the potential cause of the anorexia.

Diagnosis

Anorexia is a non-specific sign associated with a large number of diseases and husbandry-related problems. An approach to an anorexic chelonian should include or consider:

- a full examination;
- a review of the patient's history;
- faecal examination;
- blood biochemistry/haematology review;
- urinalysis, cytology, viral/microbiological culture, etc.;
- diagnostic imaging techniques such as ultrasonography, radiography, endoscopy and MRI.

Treatment

- Optimise husbandry and nutrition to remove factors predisposing to anorexia.
- Treat primary disease.

BEAK DEFORMITIES

(Figs 11.7–11.11, 13.1)

Aetiology

Various aetiological factors may be involved:
- lack of abrasive substrate;
- trauma;
- accelerated growth (protein excess);
- metabolic bone disease (MBD);
- potentially hypovitaminosis A and other metabolic disease;
- localised infection;
- osteomyelitis secondary to chronic stomatitis.

Clinical signs

- Beak lesions are often self-evident.
- Metabolic bone disease often occurs concurrently with other nutritional disorders.
- The rhamphotheca is often excessively long.
- The mandible may show deformity similar to mammalian rubber jaw with hyperparathyroidism and nutritional bone disease or lytic osteomyelitis.

History

Variable depending upon aetiology. The muscles of the jaw often distort the beak if metabolic bone disease results in inadequate calcification during periods of growth. Such animals often show a short, undershot lower law.

Diagnosis

Lesions are usually self-evident. Underlying diagnosis revealing possible cause requires more detailed investigation:

Fig. 13.1 This African spurred tortoise (*Geochelone sulcata*) has been sedated to facilitate beak burring.

- consider the history;
- assess nutrition and husbandry;
- assess haematology and blood biochemistry values.

Treatment

- Correct the causes of systemic disease and optimise husbandry practices.
- Treat underlying infections according to organisms identified and their antimicrobial sensitivity patterns.
- Trim overgrowth of the beak with a high-speed burr and abrasive disc (e.g. Dremmel and diamond cutting end). No general anaesthetic is required for this.
- Beak-repair materials marketed for avian use may help where reconstruction of facial anatomy is necessary. Epoxy resins may help stabilise fractured and splintered beak material.
- External fixators may assist in the stabilisation of mandibular fractures.

CLOACAL ORGAN PROLAPSE

(Figs 11.97–11.103, 13.2–13.18)

Aetiology

- Some degree of intermittent cloacal-organ exposure is normal in both sexes after sexual maturity is attained. Normal males will

Fig. 13.2 Replacing a cloacal organ prolapse.
In order to preserve viability, a contaminated prolapse should be cleaned, examined and where possible reduced at the earliest opportunity. If the animal must be transported to a veterinary surgery by a keeper, the prolapse can be rinsed under running water and wrapped in clean plastic food wrapping.

Fig. 13.3 Blunt instruments, digits, rubber stomach tubes and gentle water pressure can be used to invaginate or invert structures such as oviduct, bladder or rectum that have become intussuscepted or everted.

Fig. 13.4 Water and lubrication were used to reduce this everted oviduct slowly.

occasionally protrude their copulatory organ, especially when handled or when urinating or defecating. Mature females also have a cloacal organ, and should this become regularly visible it may be referred to as clitoral hyperplasia by some clinicians.

- Persistent exposure of the cloacal organ of either sex may be an indication of low calcium status, parasitism, or other problems, especially if concurrent with other signs of disease.
- Prolapse of the cloacal organ, where self-correction and organ withdrawal is no longer possible, is associated with a variety of clinical situations.

Potential causes and predispositions include:

- general debility;

Fig. 13.5 Using retractors to increase the size of the cloacal orifice is helpful.

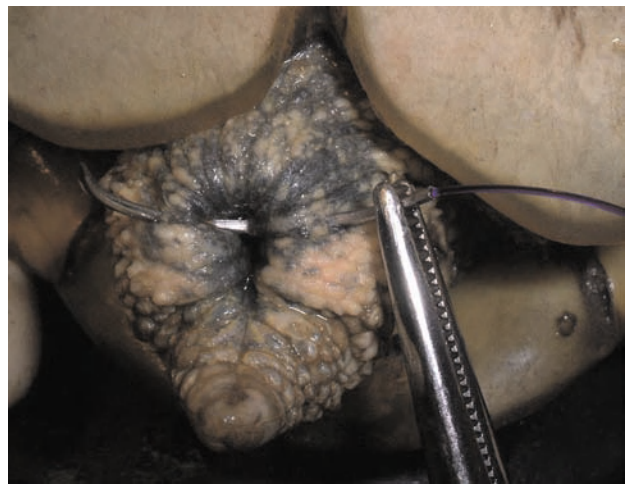

Fig. 13.6 A temporary purse-string suture can be used to retain the cleaned and replaced prolapse.

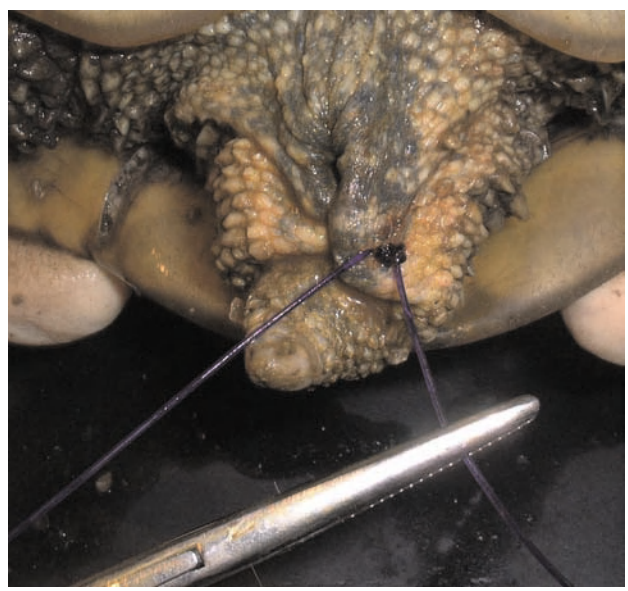

Fig. 13.7 It is important that a purse-string suture allows elimination of urine and faeces, but ensures that the prolapse is retained. It may be necessary to slacken or even replace the suture regularly in order to allow appropriate cleaning and checking of cloacal structures.

Fig. 13.8 Removal of a necrotic penile prolapse.
The organ is pulled forward until healthy tissue is revealed. A mattress suture (PDSII®, Ethicon) is placed through healthy proximal penile material to achieve haemostasis.

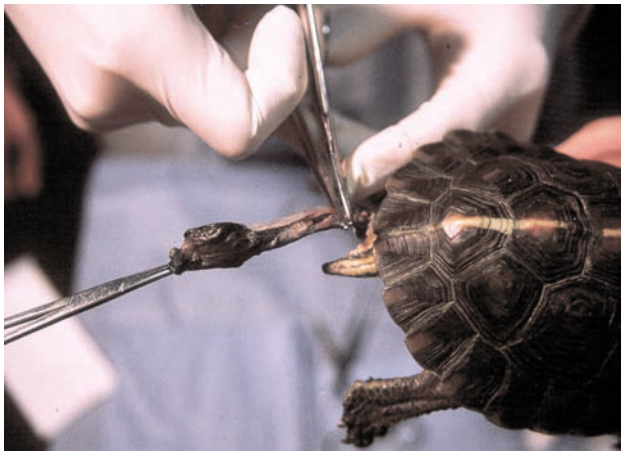

Fig. 13.9 Devitalised tissue distal to the suture is then removed surgically. Post-operative analgesia and antibiosis are normally provided.

Fig. 13.10 Management of a necrotic oviductal prolapse.
Application of paired haemostats at the base of a necrotic and traumatised chronic oviductal prolapse. The clamps were placed as proximal as possible in order to ensure removal of all devitalised tissue.

Fig. 13.11 A transfixed crushing suture was placed around the base of the prolapse which was then removed with scissors.

Fig. 13.12 After the contaminated prolapsed material was removed and discarded, it was possible to exteriorise the healthy oviduct remnant to allow suture placement.

Fig. 13.13 The stump is over-sewn using PDS. Before being allowed to fall back into the cloaca and returned into the coelomic cavity.

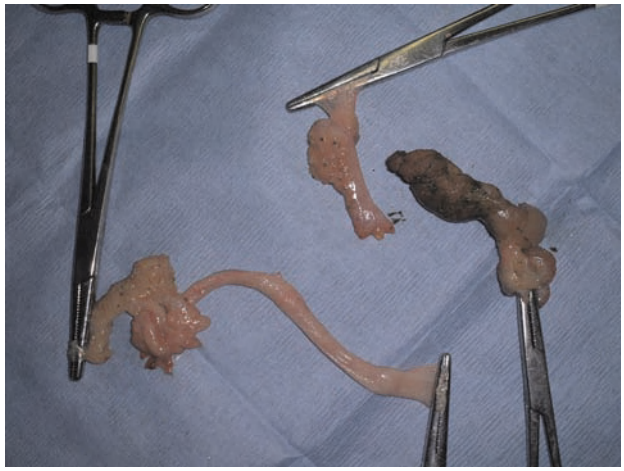

Fig. 13.14 The other oviduct, the remnants of the oviduct in question, and the two ovaries were removed through a coeliotomy. It would be unwise to leave such material within the animal.

Fig. 13.15 Once reversed, the prolapsed structure looks more typical of oviduct.

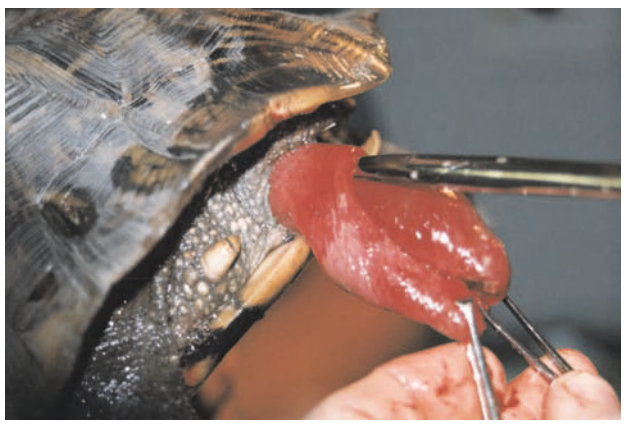

Fig. 13.16 This female *Testudo graeca* suffered a major prolapse. The exact organ was not identified. Unfortunately the organ was severely compromised and had become friable and necrotic. The patency of the cloaca was ensured by careful examination and location of important orifices using blunt instruments.

Fig. 13.17 After appropriate cleaning, circumferential mattress sutures were placed around the prolapse.

NB: The authors advise the routine use of surgical gloves where possible.

Fig. 13.18 The prolapse was removed distal to the sutures and the tortoise made an uneventful recovery, followed up over the following two years. As it is likely that the structure removed was an oviduct, it is unknown if the ovary on the affected side will become atretic and inert or may compromise health at some future point. Therefore this procedure can only be considered as a salvage technique.

NB: The authors advise the routine use of surgical gloves where possible.

- neurological dysfunction;
- any coelomic space-occupying lesion or other cause of straining (e.g. dyspnoea, constipation, egg retention, oviposition, cystic calculi, etc.);
- metabolic disease (e.g. hypocalcaemia, ketoacidosis, hyperoestrogenism and secondary cloacal hypertrophy);
- obesity;
- excessive libido;
- bacterial, fungal, viral and parasitic infections of the lower genitourinary and digestive tracts.

Some factors predispose specific structures towards prolapse:

- bladder—urolithiasis, eggs within bladder;
- shell gland/oviduct—egg retention, salpingitis;
- penis—infection, mating injuries or other trauma, forced separation during copulation, substrate contamination, ground contact by engorged organ.

Clinical signs

- A structure prolapsed through the cloacal opening.
- Hypocalcaemic animals may have soft shells if disease is chronic or they are juvenile. Such animals may be weak or displaying paresis.

History

- Depends upon the structure.
- Males are often presented during periods of sexual activity, which may coincide with recent periods of good weather, or recent exposure to females.
- Female chelonians often prolapse oviductal material during dystocia. Some females may have partially laid a clutch of eggs.

Diagnosis

- The prolapsed organ should be identified wherever possible by endoscopy, digital examination, visual identification and histopathology.
- A full examination of the animal, including blood parameters and radiographs should be performed, as the animal may well be systemically ill.
- Faecal examination is also indicated.
- The history may suggest calcium metabolism problems.
- Male animals may have been sexually active in the recent past.
- Female animals warrant radiographic examination for the presence of eggs, and ultrasonography for assessment of follicular activity.

Treatment

An appropriate treatment protocol depends upon the aetiology and the degree of secondary change in the prolapsed structure. Retention of the reduced organ using a purse-string suture, and correction of any husbandry or nutritional problems, will sometimes suffice. Knowledge of what has prolapsed is required in order to determine the form any necessary amputative surgery must take.

- Determine what conditions may have predisposed to the prolapse, and correct these in order to prevent continued disease of this or other organs. Metabolic diseases will need corrective management. Husbandry problems will need the keeper to be given appropriate advice for future correction.
- Surgery may be necessary to reduce a prolapse, or, if it has become heavily infected or necrotic, to remove it. It is common to amputate uterine horns and penile prolapses where trauma is extensive. Excellent results are possible in both cases provided that the patient is appropriately stabilised and treatment is commenced in time. A surgical approach to the management of cloacal-organ prolapse is given in the Surgery section of this book.
- Excessive libido in male animals may be reduced by alterations in photoperiod and temperature provision. The use of anti-androgens and other libido-decreasing hormones has not yet been investigated.

CUTANEOUS AND SUBCUTANEOUS LESIONS

(Figs 11.25–11.46, 11.65–11.72)

Aetiology

The areas of chelonian skin traditionally covered by 'T-shirt and shorts' in man experience increased friction in comparison to limb extremities, and may therefore exhibit exaggerated lesions when skin abnormalities are present. Common causes of dermal lesions include:

- nutritional disease (e.g. hypovitaminosis A or hypervitaminosis A);
- obesity, which may lead to extensive or localised fatty deposits;
- bacterial infection (e.g. subcutaneous abscess/fibriscessation or cellulitis);
- septicaemic cutaneous ulcerative disease (SCUD);
- viral infection, fungal infection, bacterial infection;
- sub-scute infections may be bacterial or mycotic;
- burns;
- ectoparasites (imported animals may have ticks or bot-fly larvae);
- vascular disturbance to dermal bone (e.g. from a prior crush injury or exposure to excessive basking heat source);
- chemical irritation/excessive use of bleaches or other disinfectants;
- inappropriate humidity or temperature;
- damage from abrasive enclosure substrate/poor construction of enclosure;
- confinement of inactive species with inappropriately managed heat sources (heat-mat blisters etc.);
- metabolic disease resulting in immobility or immunocompromise and infection;
- heat/frost necrosis;
- fall injury;
- car trauma;
- rotary mower injury;
- dog/rat bite trauma;
- self-trauma (e.g. males where libido is excessive and animals thrash against enclosure sides).

Clinical signs

Various dermatological presentations of disease are illustrated in the accompanying plates.

- Clinical signs vary with aetiology.
- Many animals will be suffering from more than just skin disease, and clinical signs may therefore be related to concurrent systemic disease.
- Animals with skin infections are also likely to have septicaemia and so there may be evidence of joint or other systemic infections.

History

History will vary with aetiology.

Diagnosis

As there are various causes, diagnostic options are extensive and should address the general health of the animal as well as the specific lesions observed. Options include:

- history review;
- clinical examination;
- clinicopathological evaluation (haematology, blood biochemistry, cytology, culture, etc.);
- radiography may give evidence of joint infection if skin infection has disseminated;
- biopsy, culture or cytological examination of skin lesions as described in the Clinical Pathology section of this book is usually helpful in formulating a therapeutic management plan;
- viral disease may require molecular tests (PCR), electron microscopy or other appropriate methods of viral identification.

Treatment

Treatment varies with aetiology and concurrent disease. The Therapeutics section and various other sections of this book discuss management protocols for various skin lesions.

CYSTIC CALCULI

(Figs 11.99 & 13.19)

Aetiology

- Cystic calculi are often the product of dehydration, especially in uricotelic species.
- Metabolic derangements such as acidosis and alkalosis, hepatopathy and unsuitable mineral balance of the diet or water source may also be predisposing causes.
- Moderate sodium, potassium or ammonium urate precipitation may be normal in uricotelic species, calcium urate might be more likely with over-supplementation.

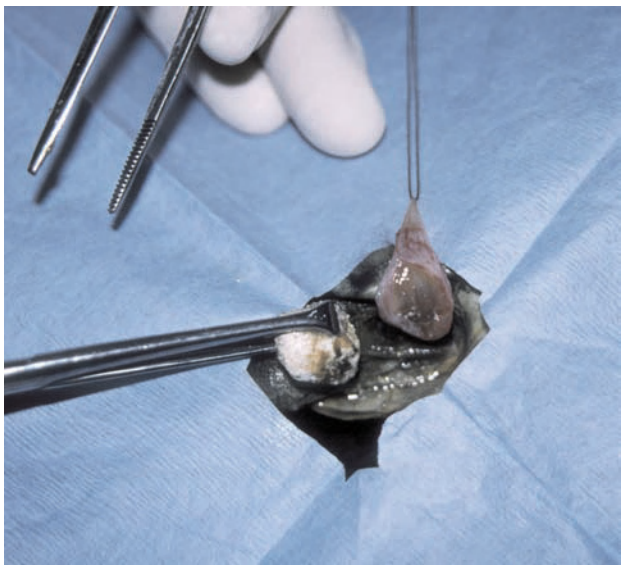

Fig. 13.19 Cystotomy and removal of a bladder stone through a soft tissue approach through the inguinal fossa of a juvenile *Testudo hermanni*.

Clinical signs

Possibly none; alternatively, any of:

- dysuria, anuria, posterior paresis, increased weight, coelomic enlargement, distension of the prefemoral fossa;
- tenesmus and/or straining, repeated lifting of the caudal plastron;
- dystocia and/or cloacal organ prolapse may result.

History

- Occasionally cystic calculi are an incidental finding.
- Possibly restricted access to fluids, recent hibernation, over-supplementation, etc.
- Animals may have been observed to be dysuric or exhibiting tenesmus.

Diagnosis

Calculi must be differentiated from ectopic eggs.

- Radiography (especially the dorsoventral view).
- Ultrasonography.
- Exploratory coeliotomy.
- Cloacal endoscopy.

Treatment

- May be an incidental finding. Ascertain if responsible for any clinical signs.
- Correct underlying cause.
- Attempt dissolution if patient is stable.
- Bladder uroliths may require surgical removal, especially in gravid females presented with dystocia. Consider coeliotomy and removal if thought to be clinically significant.
- Juvenile hatchlings commonly present with large deposits of what appears to be calcium or ammonium urate in the bladder, often putty-like. In one such case, lithotrypsy was attempted, but without success (Calvert 1998: personal communication).
- At our surgery we have attempted stone dissolution and/or removal by bladder irrigation, and cloacal removal. Plastral coeliotomy is indicated if the animal is distressed with repeated straining to urinate (stranguria) and increased frequency of urination (pollakiuria).

DIARRHOEA

Aetiology

It is unclear whether many animals presented with diarrhoea are suffering from a true clinical condition. Moist, bulky stools may be the product of the normal excretion of urine mixed with fibre-rich faeces. Clinically-significant diarrhoea is relatively uncommon.

Possible causes of diarrhoea include:

- unsuitable/inappropriate food intake: dietary intolerance (e.g. excessive fruit/sugar);
- intussusception (although this may be a consequence as opposed to a cause);
- parasitism (helminth/protozoan);

- enteritis/colitis;
- septicaemia;
- toxaemia;
- yeast overgrowth;
- unbalanced gut flora (following recent antimicrobial therapy);
- inappropriate environment (e.g. lack of basking area, too cold, too hot, etc.);
- stress—transportation, handling and examination;
- metabolic imbalances;
- mycotic and viral disease.

Clinical signs

- Excessive, bulky, moist, inadequately-digested/processed faeces.
- If animals become dehydrated or hypokalaemic, then weakness, anorexia and inactivity.

History

- Varies with aetiology.
- May be normal in summer if fluid intake is good.

Diagnosis

- Full examination of the patient, husbandry and diet.
- Faecal examination (wet smear, floatation, sedimentation, culture).
- Guides to the identification of agents that may cause intestinal disease are given earlier, in the section describing faecal examination in Clinical Pathology.
- Faecal microscopy should be undertaken in any animal suspected of having diarrhoea.
- Similarly, a guide to normal chelonian gut flora has also been given earlier, and the reader is referred to this should microbiological culture be undertaken.
- Blood biochemistry and haematology parameters should be assessed.

Treatment

Depends on aetiology and predisposing factors:

- Nutritional support, based around a high-fibre diet, is probably indicated in most instances, along with fluid support and possibly potassium supplementation.
- Probiotics (e.g. back-feeding faeces from a healthy colony mate) may help stabilise gut flora.
- Antiparasitic and/or antimycotic treatment may be indicated if faecal examination suggests the presence of pathogenic organisms.

DYSTOCIA

Aetiology

Dystocia in a chelonian can be defined as 'a failure to deposit eggs within a time considered usual for the species concerned'. This is often a subjective assessment. According to Lloyd (1990), dystocia occurs in 10% of the captive reptile population annually in the United States. Raiti (1995b) puts this figure at 9%.

The presence of calcified eggs is not necessarily an explanation of any illness, and shelled eggs in the coelomic cavity do not necessarily require treatment. They may merely reflect normal gravidity in a normal animal. Radiographic or ultrasonographic observation of shelled eggs within female chelonians is often a normal incidental finding.

Chronic retention of eggs is likely to progress to more serious disease, but it is not clear exactly when and how this will happen. Many healthy, captive *Testudo hermanni* appear quite happy to ovulate in the autumn, retain their eggs throughout a five month hibernation, and then lay fertile eggs within a few days of emerging from hibernation (Harcourt-Brown 1999). Little published data is available on normal egg production and retention times (gravidity/gestation periods) in most species in the wild or in long-term captivity. This makes it very hard for a clinician to be completely sure whether things are going well, or whether dystocia is present.

A full clinical examination and review of the history are indicated whenever a sick chelonian is presented and the possibility of disease unrelated to any observed/discovered/presumed gravidity should be considered. If a gravid female chelonian is displaying signs of ill health, then disease of non-uterine origin may have prevented this animal from laying its eggs. Persistence of coelomic eggs may be an indication of systemic disease or inappropriate husbandry. Resolving such problems is integral to the appropriate treatment of the dystocia.

Occasionally, chronic egg retention (e.g. because of abnormally large or misshapen eggs) will become a significant cause of disease. In such cases the uterine contents are often putrid and the situation comparable to mammalian pyometra. Holt (1979) describes egg retention in a *Testudo graeca* associated with a *Proteus* and *Pseudomonas* infection of the cloaca. This appeared to ascend and result in a salpingitis.

Rosskopf & Woerpel (1983) suggest that coelomitis, metritis, uterine or bladder rupture and shock may result from untreated dystocia in chelonians. It is this author's (SM) experience that chelonians presented with oversize or abnormal eggs within the pelvic canal are often unable to urinate or defecate as a result of cloacal obstruction. In such cases, faecal material is forced up into the uterine horns, resulting in profound and debilitating metritis.

Multiple aetiologies for dystocia are possible and these may include:

- inadequate nesting site provision;
  (1) competition for nesting sites,
  (2) intra-/inter-species aggression,
  (3) inability to exhibit nesting behaviour due to stocking density,
  (4) disturbances from aggressive males.
- inappropriate environmental provision (according to Lloyd (1990), failure to provide a suitable nest site, with a suitable substrate at a suitable temperature, is a major cause of dystocia in captive breeding females.);
  (1) too hot,
  (2) too cold,
  (3) too dry,
  (4) too humid,
  (5) too dark,
  (6) too bright.
- physiological and morphologic abnormalities; systemic illness;

(1) mechanical obstruction (abnormally large eggs), abnormally shaped eggs,
(2) obstruction of egg passage by bladder deposits,
(3) reproductive tract infections,
(4) systemic illness (hypocalcaemia, hypokalaemia, dehydration, etc.),
(5) endocrinopathy,
(6) ectopic eggs.

Some keepers suggest that inappropriate handling of gravid females, especially tipping them onto their backs, which predisposes to displacement of eggs into the bladder, can cause dystocia. This unusual form of dystocia is unresponsive to medical and environmental modification: a cystotomy is required.

Clinical signs

No presenting signs are pathognomonic for dystocia in chelonians. Gravidity may be an incidental finding of radiographic or other examination. It is important to determine if the presence of coelomic eggs has clinical significance during the work-up of any case.

Dystocia may become apparent when gravid females exhibit complications, such as yolk coelomitis (egg coelomitis), straining or prolapse of oviductal structures.

Early stages

- In the early stages there may be no signs.
- The duration over which gravid females can carry eggs normally before oviposition is poorly defined.

Chronic Dystocia

As the animal's health becomes compromised through systemic disease predisposing to dystocia, or dystocia itself, various signs are possible including:

- abnormal posture;
- hind limb paresis;
- anorexia;
- lethargy;
- inactivity;
- continuous or repetitive straining;
- malodorous cloacal discharge;
- faecal and/or urinary retention;
- cloacal organ prolapse;
- involuntary elimination of urine over a period of several days.

History

- The change from gravidity to dystocia is not often associated with specific history.
- Animals often experience poor husbandry and nutrition.
- There may have been a failure to provide a suitable, isolated nesting area.
- Concurrent disease may be present.
- Most animals will have been in intimate contact with a male within the previous four years.

Diagnosis

Dystocia must be differentiated from normal ovulation, gravidity and production of shell within the oviductal shell gland. This is also discussed later in the section covering the surgical management of dystocia. The appearance of normal eggs, retained eggs and ectopic eggs within the bladder is illustrated elsewhere, in the Radiography section (Figs 8.48, 8.49, 15.63–15.67).

Examination of cloacal discharges or cytological examination of a coelomic effusion may suggest that salpingitis or yolk coelomitis is present (Frye 1991a). He also suggests that the possibility of yolk coelomitis is a justification for coeliotomy and egg removal in chelonians. There is urgency in this if irreversible terminal pathology is to be avoided.

Diagnosis of gravidity is often achieved radiographically, but further useful information facilitating a diagnosis of dystocia is also available from a variety of other examination methods:

- presenting signs;
- history;
- weight;
- ballottement;
- cloacal examination;
- radiography;
- ultrasonography;
- laparoscopy;
- cytology of coelomic effusions and cloacal discharges;
- further assessment of systemic health including blood parameters.

Treatment

It is only necessary to treat dystocia, and not normal gravidity! The distinction between the two entities may be blurred, and one can often be misinterpreted as the other. It is hoped that the distinction between these two states will become clearer as a database of information regarding normal captive chelonians is built up. Treatment is always advised where the diagnosis of dystocia is relatively clear-cut.

Where a repeated failure of medical management has occurred, or radiography reveals egg abnormalities or ectopic eggs (e.g. within the bladder), elective coeliotomy is indicated.

Provide a suitable nesting area

When illness or obstruction is not involved in a dystocia case, improvements in the environment may encourage egg-laying behaviour. This may be straightforward if animals are housed in natural climates, but may not be possible where latitudes and captive environment are unsuitable for the species.

Nesting areas should always be provided when mature female tortoises or turtles are kept.

Overcrowding, and housing with unsuitable animals should be corrected. Gravid animals that have not been observed to have laid their eggs should be reassessed regularly in order to ensure that they do not progress to systemic illness.

Even if males are not present, females may still lay eggs. It is possible through sperm storage that such eggs may even be fertile, especially if the chelonian has been recently taken from the wild.

Nesting area provision

The following are criteria for a suitable nesting site:

- A nesting area should be at least four to five times larger than the carapace area of the female.
- Substrate should be about twice as deep as the carapace is long.

- Slightly moist potting soil or sand is a suitable substrate and a plastic dustbin on its side is a suitable nesting chamber.
- Surface and depth temperature will influence choice of site.
- The ground should be heated appropriately (e.g. 30°C surface temperature with some Mediterranean tortoises).
- Most chelonians lay eggs at night, and excessive light should be avoided. Traditionally, animals at the author's surgery are maintained in a relatively dark, warm, humid room.
- It is best for a keeper already to have offered an appropriate nesting area prior to the chelonian becoming gravid, rather than introducing it at a late stage, hoping it will be 'approved of'.

Medical induction (calcium supplementation and oxytocin injections)

Oxytocin in conjunction with β-blockers, fluid therapy and baths to improve hydration, and calcium supplementation (where deficient), have been used to induce oviposition with great success.

Concurrent calcium administration is suggested in many texts to improve contractility of oviducts. Such supplementation is probably appropriate if animals have not been provided with food containing appropriate Ca:P levels or adequately supplemented with a calcium/vitamin D balancer, or where full-spectrum lighting has not been made available to a basking species.

Calcium administration by injection is not appropriate for a dehydrated patient or an animal with electrolyte imbalance. Pre-treatment assessment of routine blood parameters including calcium (ionised and bound if available), potassium, and PO_4^{3-} levels is advised. Oral supplementation and stabilisation may be more appropriate in many cases.

Raiti (1995b) suggests that uterine contractions do not improve with calcium therapy unless a hypocalcaemia is present, but hypocalcaemia appears to be relatively common in poorly maintained females presented at this author's surgery.

Suggested induction protocol

Soak the tortoise or turtle in a warm, shallow bath for a period in excess of ten minutes on a regular basis during stabilisation. The author (SM) would bathe a hospitalised dystocic chelonian twice daily, and consider several days of hospitalisation and stabilisation prior to induction of debilitated cases.

Some authors suggest that oxytocin is less effective if uterine tissue is inadequately hydrated. As dehydration is so hard to diagnose, it is probably wise to give active fluid therapy prior to induction (e.g. 1% body weight/day in ml for two or more days prior to oxytocin).

Cloacal lubrication with warmed obstetric lubricant is helpful. (Try to do this before giving any medication).

Prepare a suitable egg-laying environment (e.g. a dustbin tipped on its side, filled with soil/sand mix, or a sheltered and private maternity vivarium).

Provide appropriate heat and humidity (a dark, warm and humid environment is often well received).

The effects of oxytocin appear to be potentiated by prior administration of β-blockers such as atenolol and propranolol. These may relax a primitive functional cervix at the base of the oviducts (Gross *et al.* 1992). Dosing is empirical, but propranolol was used with success by Gross *et al.* (1992) in lizards at a dose of 1 μg/kg body weight orally. This author has used atenolol (Atenolol Ph Eur 25 mg tablets, CP Pharmaceuticals, Wrexham, UK) at 7 mg/kg orally in *Testudo* spp. and red-eared sliders (*Trachemys scripta*) and other reptiles with great success.

If it appears that the patient is hypocalcaemic (normal ionised calcium values are poorly defined but are generally >1 mmol/l), then calcium gluconate or borogluconate (10% solution) can be given at 0.5–1.0 ml/kg IM/SC, to complement any oxytocin given later. Where this solution is used, it is wise if it precedes the oxytocin by several hours. The author tries to give calcium in the evening and then oxytocin the following morning. Avoid giving calcium salts to an azotaemic, hyperuricaemic or dehydrated reptile (gout, renal failure and soft tissue mineralisation may occur).

Oxytocin at 1–3 IU/kg intramuscularly is generally effective. The intraosseous route is also suitable using an infusion (Divers 1997b). Intraosseous infusion is suggested to be more effective than bolus injections. If egg expulsion has not occurred following a single oxytocin injection, 50%–100% of the initial dose may be repeated intramuscularly four to twelve hours later.

This author (SM) is prepared to continue this protocol in combination with fluid therapy, and β-blockers whilst eggs continue to be produced at a rate greater than one every 24 hours or so. Alternatively, if egg production ceases despite repeated dosing, the patient should be carefully reassessed for other problems, such as relative egg oversize.

If the health of the dam is good, and this initial attempt to induce ovulation over one or two days fails without any obvious reason, it may be wise to delay any further attempts at induction for ten days. Maintain hydration and environmental conditions suitably during this period. Exhausted and debilitated animals may need appropriate stabilisation and attention to any causative factors.

Prostaglandins may prove to be of use in medical induction and appear to induce nesting behaviour.

Surgical treatment of dystocias

Salpingotomy

Salpingotomy is an appropriate treatment for a confirmed dystocia that is repeatedly unresponsive to medical therapy, or where it is obvious that medical therapy will be ineffective (e.g. with egg abnormality or oversize). Adequate funds and facilities must be available for such a procedure.

Detailed information on procedure is given in the Surgery section of this text (Figs 15.66–15.71). Fluid therapy, antibiotics and analgesics should all be carefully considered preoperatively. Surgery usually involves an approach via a transplastral osteotomy/coeliotomy, although the prefemoral approach can be utilised in large individuals, where the plastron is relatively small in comparison to the carapace.

Where a plastron osteotomy approach is utilised, eggs can be removed easily through oviductal tissue elevated to the surgical site. Eggs may be elevated to the coeliotomy site by using finger holes in the handles of surgical equipment, or sterile teaspoons.

Occasionally individuals are simultaneously spayed during salpingotomy. It may be wise to consider ovariosalpingectomy in order to prevent future reproductive disease, or where uterine contents are degenerate and necrotic.

Generally, salpingotomy carries a good prognosis if the patient is appropriately stabilised and nursed.

Cloacal ovocentesis

Cloacal aspiration of the contents of one or more retained eggs, or ovocentesis, may be a viable salvage procedure in a dystocia case. Such aspiration is particularly applicable in cases that do not respond to medical therapy, or where a client is unable to afford coeliotomy or perhaps where appropriate facilities for salpingotomy are not available (Rosskopf & Woerpel 1983). This author (SM) has found that cloacal aspiration of eggs in cases of chronic egg retention is relatively straightforward, and gives excellent results in the majority of cases.

Following relief of any cloacal obstruction, a combination of propranolol and oxytocin is given to aid expulsion of eggs and material. A β-blocker may assist in dilating the proximal cloaca and providing improved access to eggs and visualisation via a cloacal speculum. The procedure used by this author is illustrated in the Surgical section of this book (Figs 15.63–15.65 & 15.72–15.81).

Where an egg obstructs the pelvic canal, it is possible that it acts as an obstruction to the passage of urine and faeces. Where faeces cannot be expelled, it may become redirected forwards into the uterine horns and around the retained eggs. Obstruction of urine outflow may result in depressed, toxic, and hyperuricaemic animals. These complications tend to resolve quickly, within a week or so, following relief of the obstruction, if the case is viable.

Surgical procedure

- A cloacal speculum improves visualisation of obstructed eggs. This author has satisfactorily used both a rabbit laryngoscope and an auroscope.
- Rosskopf & Woerpel (1983) used an 18G × 2″ needle and a 5 or 10 ml syringe to aspirate the egg contents in a box turtle, and this is suitable for most moderate-sized species.
- Aspirated egg/uterine contents may be grossly necrotic or infected. It is wise to check for evidence of yolk coelomitis at the time of surgery and over the following weeks.
- This author irrigates the cloaca and uterus following aspiration of egg material and manual removal of available debris. A three-way tap attached to a flushing syringe and tubing is suitable, and is illustrated in the accompanying plates.
- After aspiration of the contents of cloacal eggs, the cloaca should then be flushed with antibiotics or disinfectant solutions (e.g. povidone-iodine).
- Expulsion of debris may be assisted by the use of β-blockers, oxytocin and lubrication.

According to Raiti (1995b) the presence of oviductal adhesions to shell fragments may subsequently necessitate salpingotomy.

EAR INFECTIONS

(Figs 11.22–11.24, 13.20–13.32)

Aetiology

Most chelonian ear infections appear to be the result of an infection ascending the eustachian tube. Any species or chelonian type may develop an ear infection. Infection may spread by the haematogenous route and result in septicaemia or abscess/fibriscess formation within other organs or structures. This is more likely than the reverse scenario, which is also possible, as the ear has connections with the outside world and is an obvious entry point for infection, especially where husbandry and nutrition are poor.

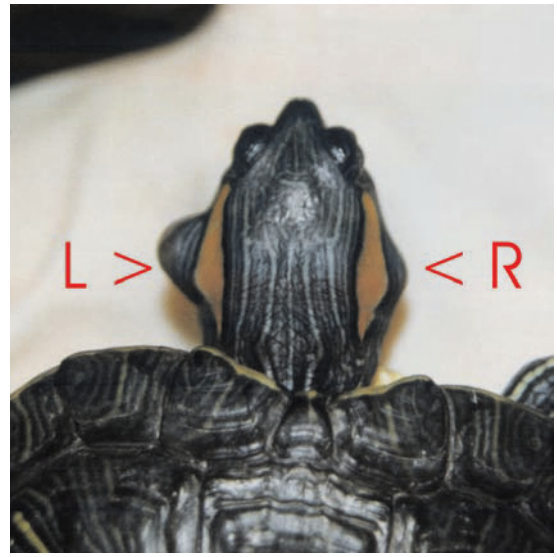

Fig. 13.20 Bilateral tympanic/ear abscess in a red-eared slider (*Trachemys scripta*) (DV).

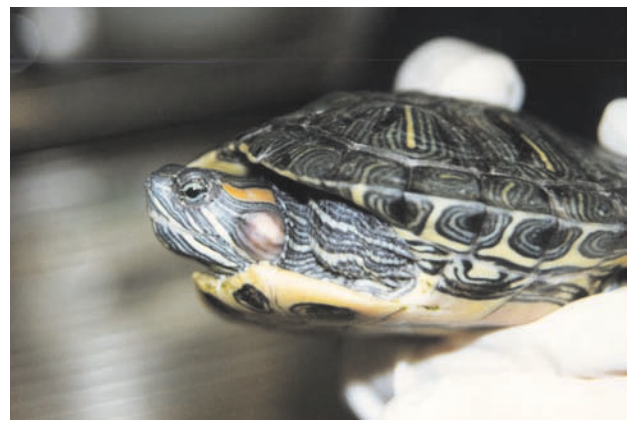

Fig. 13.21 Tympanic/ear abscess in a red-eared slider (lateral).

Concurrent disease and debility are common.

In most cases poor hygiene and immunosuppression (e.g. inadequate nutrition or temperature provision) are predisposing factors:

- Ear infections are more common in semi-aquatic animals where control of water quality is inadequate.
- Terrestrial tortoises maintained at unsuitably low temperatures, such as in a United Kingdom back garden, without supplementary heat or shelter, also readily develop such infections.

Clinical signs

- Swelling of one or both tympanic scales is usually apparent.
- Asymmetry of the head may be obvious, although this is not invariable.
- A discharge may be seen at the dorsolateral pharyngeal exit points of the eustachian tubes of both sides.
- An ear infection may spread and predispose to, or present as, septicaemia. They may spread to neighbouring structures, and osteomyelitis of the jaw and cellulitis ascending towards, and possibly involving, eye structures are possible.

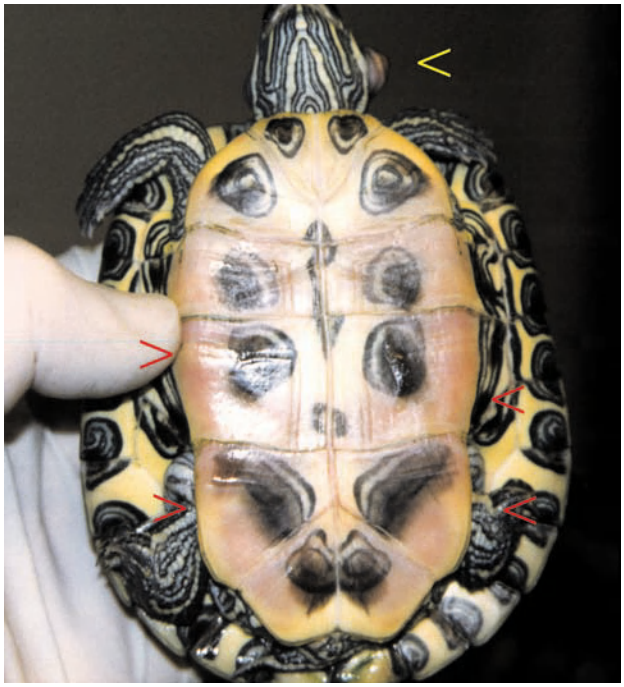

Fig. 13.22 Erythematous flush to plastron (red arrows) associated with septicaemia. This is presumably secondary to the ear abscess (yellow arrow), although poor hygiene and management may have predisposed to both.

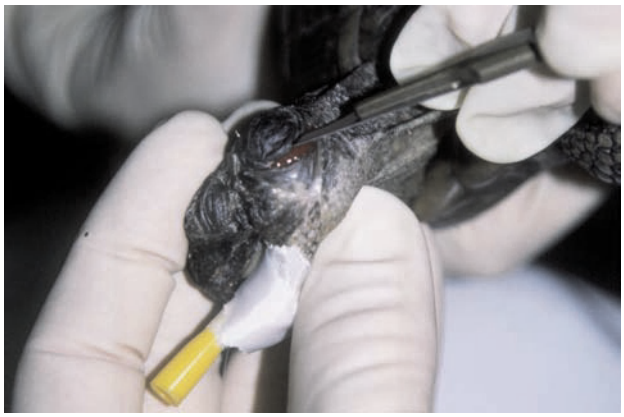

Fig. 13.23 The tympanic scute is opened around its ventral circumference (3 o'clock to 9 o'clock).

- Where infection is active, the animal may reach and claw at its head with the ipsilateral foreleg.
- The animal's balance is seldom affected in any obvious way, although balance abnormalities are possible.
- As hearing is so hard to assess in most chelonians it is unknown whether or not ear infections result in compromised hearing.

History

- The history is often non-specific; swelling may be chronic and unnoticed by the keeper.
- Ear swelling may be an incidental finding when an animal is presented for another condition, such as trauma.

Fig. 13.24 The contents of the middle ear, usually inspissated pus, are exposed.

Fig. 13.25 One or more hypodermic needles can be used to pierce the lesion and lever it out.

Fig. 13.26 Solid puss is relatively easily removed by alternating leverage upon needles. Usually the mass comes out in one complete piece with a stalk from extension into the eustachian tube.

- Recent care is often inadequate.
- Water quality may be poorly maintained in bathing species.
- Concurrent disease may be apparent.
- The animal may be presented following a recent decline, although the ear abscess is common. In such cases it is important to check for concurrent associated disease.

Fig. 13.27 The incision is generally left open, allowing further flushing of the infection site.

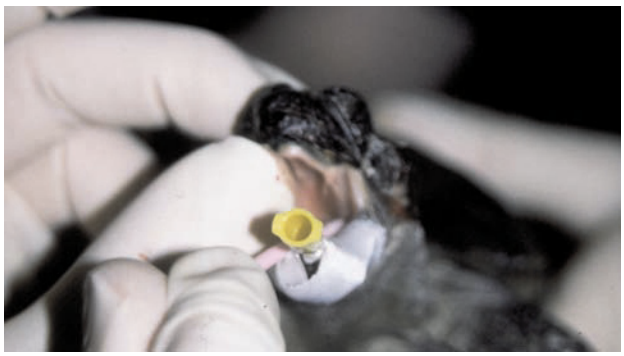

Fig. 13.28 Ensure that both ears and both eustachian tubes are checked externally and through the pharynx. A sterile cotton bud is helpful.

Fig. 13.29 Consider cytology, bacteriological culture and sensitivity testing. Local antibiotic ointments, flushing with dilute chlorhexidine solutions and systemic antibiotics are all acceptable and generally used in combination. Povidone-iodine may cause damage to fibroblasts (Murray 1996).

Fig. 13.30 Recovery is generally uneventful but recurrence is possible. This hatchling *Testudo hermanni* is shown three weeks post-surgery.

Fig. 13.31 Chronic bilateral ear abscess in *Testudo graeca* associated with periorbital infection extending into and including the nasal cavity.

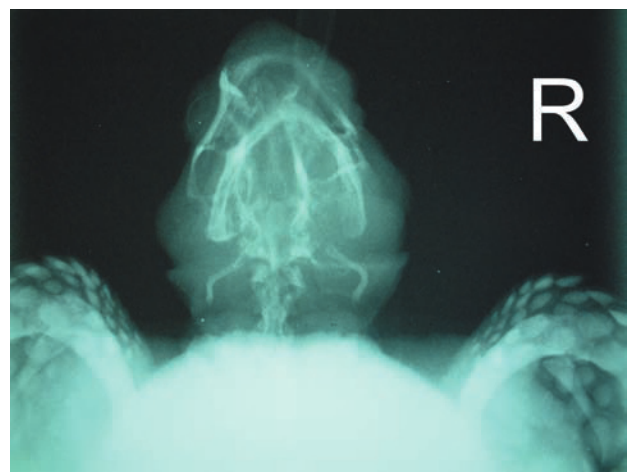

Fig. 13.32 Radiography is helpful in assessing the degree of bone and soft-tissue erosion and displacement.

Fig. 13.33 The whole of the nasal cavity is obliterated by caseous infection. The contents of the ear abscess have also been removed.

Fig. 13.34 The nasal cavity immediately after surgical debridement. There is extensive erosion of normal facial architecture, but the eye has already returned to a more anatomically normal position. This tortoise ate the same day, suggesting pain was reduced after surgery, with appropriate analgesia, and mechanical interference with prehension was reduced.

Fig. 13.35 One week post surgery.

Diagnosis

- A full clinical examination, faecal examination, and possibly a general health assessment through a blood examination may be prudent, especially if anaesthesia and surgical correction are anticipated.
- The swellings of the tympanic scutes are pathognomonic.

Fig. 13.36 Two and a half weeks post surgery.

Fig. 13.37 Four weeks post surgery.

Fig. 13.38 Anterior view of the head pre-surgery.

- Material from the ear and/or Eustachian tube should be examined. Cytology, culture and sensitivity are appropriate.
- Always check the pharyngeal exit points of the Eustachian tubes during oral examination.
- Always check to see if the condition is bilateral.
- Always look at the general health of the animal and consider the possibility of other concurrent disease, which may also need appropriate management.
- Radiography may provide evidence of osteomyelitis.

Fig. 13.39 Anterior view of head one week post surgery.

Fig. 13.40 Anterior view four weeks post surgery.

Treatment

- Treatment is usually surgical debridement, as medical treatment is unlikely to resolve the problem. Surgical treatment is described and illustrated in the surgical plates later.
- Microbiological culture and sensitivity will indicate appropriate antimicrobials to be used in the post-operative period.
- Concurrent disease should also be managed and the animal's husbandry and nutrition should be optimised.

ECTOPARASITES

Aetiology

Myiasis (fly strike) is often secondary to localised trauma and/or infection. Here, maggots infest wounds, lesions and prolapsed material. Generally, infested animals are debilitated and have significant concurrent disease.

Exotic ticks may be associated with recently imported specimens and are implicated as possible vectors of disease. This is especially true where animals are imported in large groups and mixed at reptile dealerships, without adequate control of ectoparasites (Figs 13.41–13.43).

There are increasing concerns in the United States about the importation and transfer of diseases such as heartwater (*Cowdria*

Fig. 13.41 Tick attached to the foreleg of a recently wild-caught juvenile *Geochelone pardalis*.

Fig. 13.42 Mineral oils can be applied locally to suffocate ticks. However, it is better to remove them mechanically first. If some are unreachable it's often more effective to apply an insecticide. If the ticks are infected, they are thought to release large amounts of infectious agents from their salivary glands when they are covered with mineral oil.

Fig. 13.43 Manual removal of a tick.

ruminatium) to domestic and indigenous wild animals (Burridge *et al.* 2000). Reports suggesting that ticks (*Amblyomma marmoreum*) harbouring this agent have become established in areas surrounding animal importation holding pens have led to suspension of trade in some chelonian species (*Geochelone*

pardalis, Geochelone sulcata and *Kinixys belliana*) in Florida (USDA-APHIS–(CFR pt 93)).

This author (SM) has encountered animals harbouring ticks (*Amblyomma* spp.) where various viruses were isolated including herpesvirus and a flavivirus. It was proposed that the ticks acted as a vector allowing disease transfer between individuals (Drury *et al.* 2001; McArthur 2001).

Indigenous ticks are uncommon in long-term captive chelonians in the United Kingdom, but have been presented to this author (SM). Generally they infest debilitated specimens inadequately maintained outdoors and left unobserved for long periods.

Clinical signs

- The presence of maggots or ticks.
- Concurrent disease is both possible and likely with a maggot-struck animal. Clinical signs are dependent upon what else is wrong within the animal.
- Explore any deep infected wound for the presence of maggots, for example deep bite wounds inflicted by aggressive males at the base of the tail of females.
- Check the cloaca with auroscope or endoscope.

Cowdria ruminatium is not believed to be pathogenic to chelonians. Viral agents such as the flavivirus described above may or may not be pathogenic to chelonians. Chelonians may merely act as a reservoir species for these agents and for other haemoparasites that are described in more detail earlier in the Clinical Pathology section of this book.

History

- Recent importation may suggest the presence of ticks.
- Animals may not have been adequately observed prior to noticing the infestation.
- General standards of care may be poor.
- Concurrent disease may be present and history and clinical signs will vary depending upon what else may be wrong.

Diagnosis

Observation of parasites will be diagnostic. However, it is important to examine the animal fully, and to review the history. Concurrent or predisposing disease is highly likely to be present, and must be managed in addition to the obvious external parasitism.

Where viral or other agents are suspected of having been transferred by ectoparasites, it may be wise to preserve the parasites for formal identification. Further investigation, such as attempts to isolate or observe agents within parasites, may be appropriate (e.g. light microscopy, microbial culture, electron microscopy or virus isolation).

Treatment

Management of underlying predispositions and concurrent disease is essential.

Manual removal of parasites can be very effective and will avoid exposure to untoward side effects of antiparasitic preparations, to which many species of chelonians appear sensitive. Ensure that all parts of the tick, including mouthparts, are removed. Reports of safe, effective use of external antiparasiticides are limited, but some examples are given below:

- Petney & Knight (1988) used dilute amitraz solution (Taktic®, Smith Klein Beecham) to eliminate ticks successfully during a natural infection. A dilution of 2 ml/litre was used, tick detachment was achieved and no adverse reactions were reported.
- In the United States, general guidelines issued for the control of ticks on imported reptiles were issued by Burridge *et al.* (2000):
  1. Products containing cyfluthrin (Cylence®, Bayer Corp, Shawnee Mission, KS), and permethrin (Permectrin-II®, Aspen Veterinary Products, Kansas City, MO) were found to be effective in controlling tick infestations, and apparently safe when applied to leopard tortoises (Burridge *et al.* 2002).
  2. Silica gel, pyrethrin, amitraz, fipronil, and carbaryl were considered ineffective in controlling ticks on tortoises.
  3. Liberal application of mineral oil, alcohol, and Vaseline® were reported to kill ticks in two to four hours.
  4. Vegetable oil was not recommended as it attracted fire ants.
- Environmental control is essential when imported ticks have become established in areas surrounding the reptile enclosures. Guidelines are given by Burridge *et al.* (2000):
  (1) If safe and legal, animal paddocks can be burned.
  (2) Bedding and transportation boxes can be bagged and burned.
  (3) Where burning is illegal, bedding materials can be bagged and left in direct sunlight where ticks are killed by exposure to lethal temperatures.
  (4) Commercial pesticides can be used to spray paddocks and scrub, but this is best undertaken in cooperation with local authorities and commercial pest control companies. Burrows as well as surface land must be treated.

ENDOPARASITES

(Figs 7.31–7.42)

Aetiology

The material here is a summary only. Extensive comment has been provided elsewhere in this book, and advice regarding the diagnosis and management of gut parasites is to be found in the earlier Clinical Pathology section, in Table 7.22 and in the later Therapeutics section. The microscopic appearances of many agents are illustrated in the Clinical Pathology figures.

Possible endoparasites include:

- coccidia;
- amoebae;
- *Cryptosporidium*;
- flagellates;
- ciliates;
- ascarids;
- *Proatractis* spp.;
- oxyurids (pinworms);
- trematodes (flukes).

Parasites may have a direct or an indirect life cycle. Some are pathogenic, and some incidental.

The normal organisms present within the chelonian digestive tract are mentioned in the earlier section dealing with the physiology and anatomy of digestion.

Clinical signs

- Often none.
- Debility and enteric signs, such as regurgitation and/or diarrhoea, are possible.

History

- Variable depending upon the infection/infestation.

Diagnosis

- Faecal examination and identification of organisms, larvae or eggs.

A comprehensive guide to the significance of faecal findings has been given earlier.

Treatment

Appropriate antiparasitic medication following identification of the infesting agent and its significance. Where the life cycle is indirect, remove contact with any intermediate hosts.

Oxyurids, ciliates, trichomonads, coccidia and flagellates
In many circumstances oxyurids, ciliates, trichomonads, coccidia and flagellates may not require treatment. Dietary alteration, hygiene measures and temperature management, such as a regular diurnal drop of around 10°C, may reduce numbers.

Cryptosporium, Proatractis spp., *amoebae and ascarids*
Cryptosporidium, *Proatractis* spp., amoebae and ascarids justify specific treatment. Appropriate therapy is described in detail later in the Therapeutics section of this book.

FOLLICULAR STASIS

(Figs 13.44–13.56)

Aetiology

Proposed mechanism of follicular stasis
It is assumed that upon ovulation, a corpus luteum is formed at the site of each ovulation. As a number of ovulations occur, it is proposed that eventually enough progesterone is produced to cause regression of other follicles within both ovaries. (Reduction in size of follicles in females where eggs are present within oviducts has been observed ultrasonographically at our surgery.) Follicular stasis may result from an inability to produce progesterone from functional corpora lutea.

Loss of social cues

Follicular stasis is presumed by this author (SM) to be the result of induced ovulation failing to occur in long-term isolated females of breeding age and condition (McArthur 2000a), although fertile ovulations are known to occur for several years after a successful encounter with a male.

Anecdotal evidence suggests that animals with follicular stasis may recently have been exposed to the company of a male after a period of prior isolation. Affected animals have often been

Fig. 13.44 Ultrasonographic examination is an excellent way of assessing follicular size and structure in most mature female terrestrial chelonians. In cases of follicular stasis, large numbers of mature follicles are usually present and an assortment of atretic and immature follicles may also be apparent. Occasionally anatomical restrictions and probe size limit effective coelomic assessment.

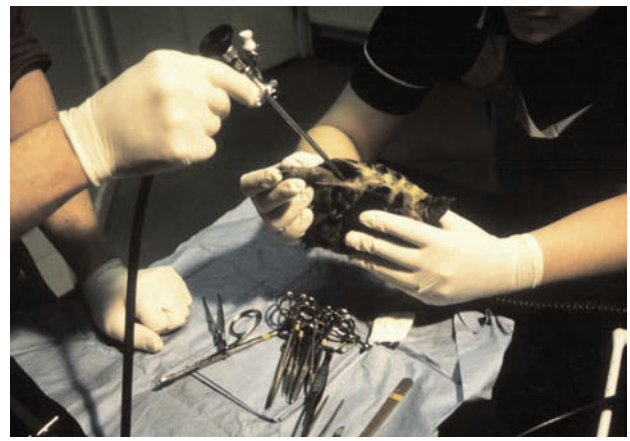

Fig. 13.45 Endoscopy helps confirm ovarian activity and gives a strong impression of whether it is normal or abnormal depending upon the number and appearance of follicles present. Great care is necessary to prevent inadvertent trauma to delicate follicles, as yolk release will provoke a severe inflammatory reaction.

Normally the endoscope is handled with two hands. This is only to show the approach.

Fig. 13.46 This ovary contains an excessive number of similarly-sized developing follicles. Ovaries like this are typical of young mature chelonians recently exposed to the temporary company of a male. Follicular development like this appears to occur in the following season(s). This presentation is relatively acute.

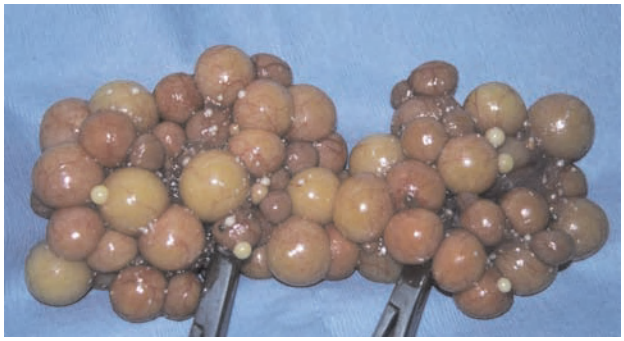

Fig. 13.47 These ovaries are a more chronic presentation than in Fig. 13.46. Here there are a variety of well-developed and oversized follicles mixed amongst degenerating atretic follicles (darker in colour) and tiny, very immature, currently-developing follicles (small and bright yellow in colour). Here the condition has been present for more than one season.

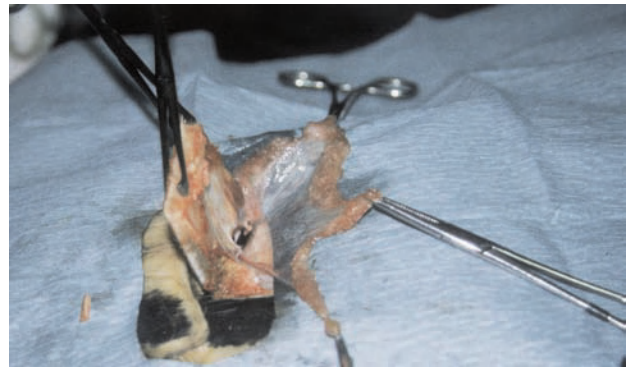

Fig. 13.50 The appearance of an inactive ovary during exploratory coeliotomy.

Fig. 13.48 The ovaries are obvious and lie either side of the bladder in the caudolateral quadrants of the coelomic cavity. They are easily exteriorised through a plastron osteotomy.

Fig. 13.51 Blunt tissue-handling instruments can be used to raise the ovaries gently. It is often impractical to pack the coelomic cavity, so great care must be taken to avoid trauma to delicate follicles. Generally ovarian tissue between follicles tolerates instrument handling well.

Fig. 13.49 A close up of an ovary in Fig. 13.48.

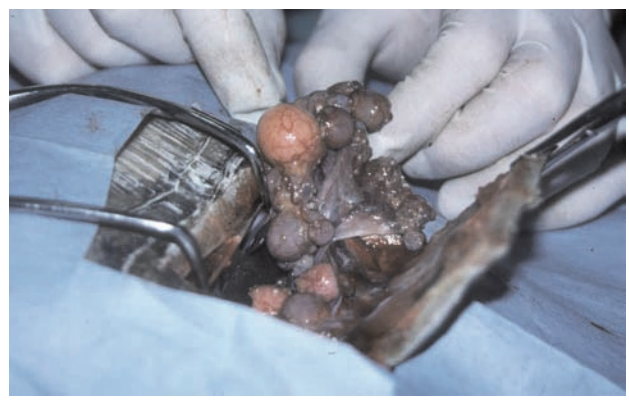

Fig. 13.52 Ovaries can be handled and raised without instruments, but this author prefers to use instruments where practical.

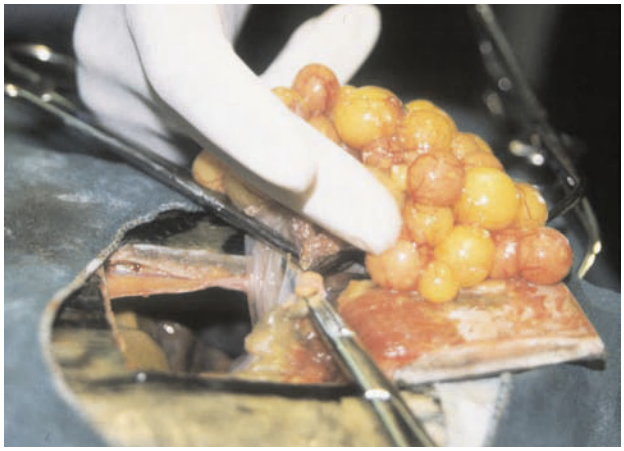

Fig. 13.53 Once exteriorised, the ovaries can be retained with a digit whilst the ovarian blood supply and mesentery is ligated.

Fig. 13.54 The ovarian mesentery has been gathered and haemostats used to clamp the blood supply and mesovarium. The liver is visible and is generally pale and lipidotic. Where vitellogenesis is still ongoing, free albumen-like liquid may be apparent beneath its capsule.

maintained with high standards of husbandry, and may have experienced good care in the immediately preceding seasons, which may in turn have encouraged the onset of reproductive activity to the point of ovulation.

Absence of environmental cues

It is possible that environmental cues such as hibernation, light and temperature are important in the coordination of both male spermatogenesis and female folliculogenesis. Animals that are not permitted to follow an annual cycle of changing photoperiod and daily ambient temperatures may lose their natural breeding cues and fail to ovulate.

In the United Kingdom, where our weather is far from suited to most reptiles, it is customary to keep tortoises indoors in enclosures with supplementary heat and light, especially during spring and autumn. Heat period and photoperiod are often maintained constantly at 14 hours heat and light, and 10 hours dark and cool. This parallels the times of waking and sleeping

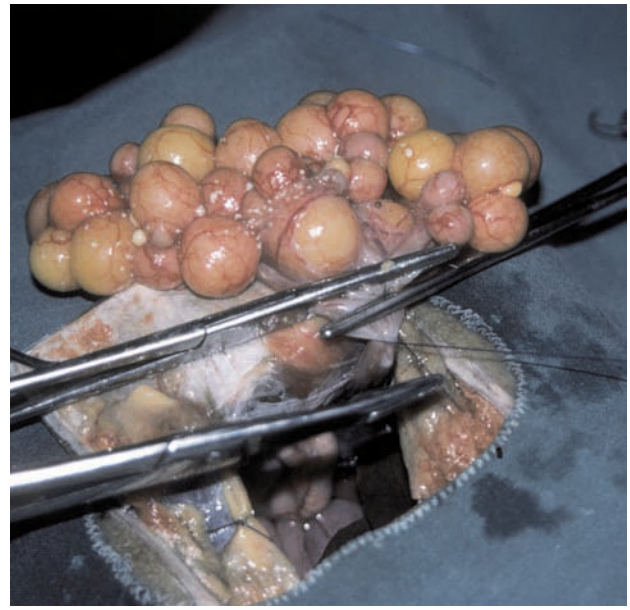

Fig. 13.55 Here the mesovarium is being ligated.

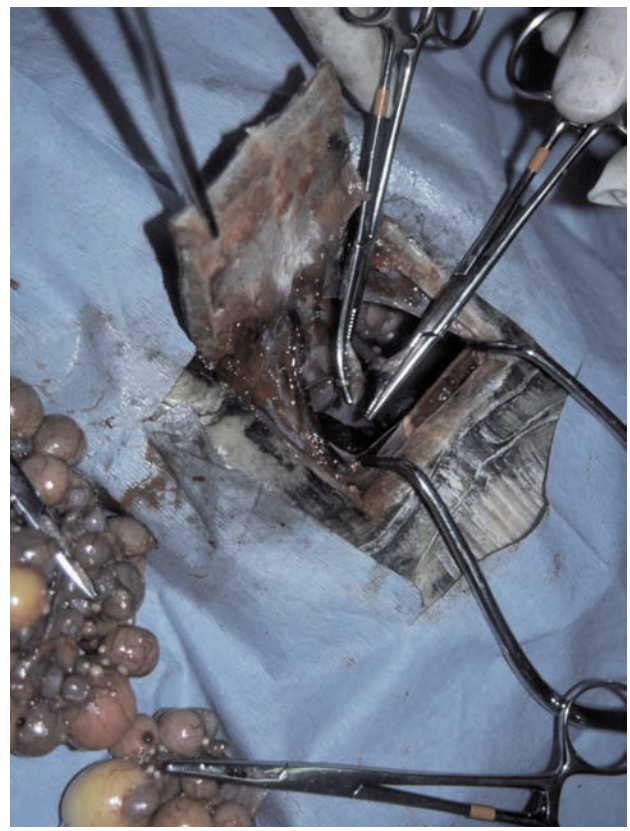

Fig. 13.56 The oviducts are returned to the coelomic cavity if preserved. Some authors advocate their removal, but long-term studies are not yet available to determine the need for it.

of the keeper. It is possible that under the influence of a human waking cycle, breeding cues are disrupted.

It is possible that an appropriate territory and nesting area must be present before chelonian ovulation may occur. If the tortoise enclosure is inappropriate, advice on improvements can be given.

Chronic nutritional disease and systemic illness
Follicular stasis in female chelonians is still in need of further research. Most females with signs consistent with follicular stasis presented at our surgery are poorly maintained. Most are kept without adequate heat, photoperiod, exposure to male tortoises or balanced nutrition. Follicular stasis may be a secondary condition when female tortoises are maintained inappropriately or acquire any chronic illness.

Clinical signs

Clinical signs appear to relate to chronicity and the degree to which hyperoestrogenism has produced metabolic imbalance (e.g. bone marrow suppression and hepatic lipidosis). Clinical signs observed by this author are vague and non-specific. Often they will be of many months' duration.

Lethargy, anorexia, abdominal distension, and weight gain have been reported in lizards with follicular stasis (Backues & Ramsay 1994; Divers 1996d). At this author's surgery, cases eventually diagnosed as in follicular stasis are generally presented with prolonged anorexia. Some cases are presented with complete anorexia and an absence of faeces for more than seven months.

Clinical signs may include:
- chronic anorexia;
- hind limb paresis;
- inactivity;
- increased weight;
- absence of faeces.

History

Cases presented at our surgery are invariably isolated, sexually mature, female tortoises. Many have male names, as over a considerable period of time the keeper has not witnessed egg laying and so has mistaken the animal's gender.

The condition also appears to be present to some extent in juvenile animals as they reach maturity, if they are maintained in isolation from males. Such animals have historically experienced good husbandry, appetite and development.

Follicular stasis is a chronic disease, and signs might arise months after onset, consequently this disease does not appear to show consistent seasonality. Cases have been presented at our surgery in summer, autumn, winter and spring.

Follicular stasis may follow temporary confinement with a male. It is plausible that follicular development is induced by pheromones or other direct behavioural interactions between animals including premating aggressions.

Diagnosis

A diagnosis of exclusion. The condition is most reliably confirmed during exploratory coeliotomy. Various things may suggest the diagnosis:
- history (as above);
- serial haematology and blood biochemistry assessment (leucopaenia, hyperalbuminaemia, hypercalcaemia);
- serial ultrasonography;
- coelomic endoscopy;
- exploratory surgery.

Haematological and biochemical findings
Hypercalcaemia (elevations in total calcium), hyperalbuminaemia, and elevations in total protein occur in females undergoing vitellogenesis. Differentiation between periods of normal follicular development and follicular stasis is not possible. If the follicular stasis is uncomplicated, other haematological and biochemical parameters may be normal.

Cholesterol and triglyceride levels may become elevated during vitellogenesis. Similar elevations also occur during hepatic lipidosis, which appears to be associated with follicular stasis (SM: personal observation).

Ultrasonography
Data on the ultrasonographic assessment of normal annual follicular cycles is limited. A summary of findings at our surgery includes:
- During late autumn and spring moderate numbers of follicles (up to ~20), 15–22 mm in diameter, are considered to be normal in mature female *Testudo* spp. presented at our surgery.
- Where the numbers of medium-sized follicles (5–15 mm) and large follicles (15–22 mm) are in excess of ~50, follicular stasis or ovulatory failure is strongly suspected.
- If sequential ultrasonography in *Testudo* spp., over several weeks, demonstrates 20–50 mature ovarian follicles 15–22 mm in diameter, follicular stasis is suggested.
- In follicular stasis, follicles do not seem to progress to ovulation or to regress and become resorbed.

Endoscopy
Endoscopic examination of mature females in follicular stasis reveals 20–50 follicles 15–22 mm in diameter. It is not easy to differentiate between normal folliculogenesis and stasis from a single examination.

An accurate count of the number of coelomic follicles is often impractical.

Exploratory coeliotomy
It is hard to differentiate between normal ovarian activity and follicular stasis without further supportive history and evidence that follicular progression was absent. Exploratory coeliotomy in cases of follicular stasis may reveal a coelomic cavity packed with 20–50 follicles 15–22 mm in diameter.

Treatment

General treatment
All aspects of husbandry and nutrition should be investigated and improved where necessary. Hepatic lipidosis is a consistent finding in cases of reproductive stasis presented at our surgery, therefore animals may be managed as though hepatic lipidosis is present. Both surgical and medical treatment have been utilised at this author's surgery (SM). Both have advantages and disadvantages. Medical management has not been reliable, though, and ovariectomy remains the author's treatment of choice.

Surgical treatment
Ovariectomy appears to be the current treatment of choice in chronic cases. The procedure is described and illustrated later in the Surgical section of this book.

Orosz *et al.* (1992) suggested follicle aspiration as a method

of treating preovulatory egg binding in a green iguana. The high probability of complications, such as egg coelomitis, as well as anatomical restrictions resulting from the chelonian shell, makes the author feel that follicle aspiration would be unsuitable in most presentations of follicular stasis.

Backues & Ramsey (1994) suggested ovariectomy for the treatment of follicular stasis in lizards. Assuming there is no intention of breeding, several authors, including Backues & Ramsey (1994), Raiti (1995b) and Divers (1996d), describe ovariectomy/ovariosalpingectomy as the treatment of choice in isolated, anorexic female lizards.

Ovariosalpingectomy in tortoises has been described by Holt (1979), Müller *et al.* (1989), Bennett (1993b) and Divers (1997a). It appears to be a suitable method of managing ovariouterine disease in chelonians. Ovariosalpingectomy is described at length in the chapter dealing with surgical conditions of chelonians.

There are potential long-term complications, such as osteoporosis, associated with spaying chelonians, but these are not yet described in the literature. This author has not witnessed such complications within the first six years of spaying.

At the author's surgery (SM) we have spayed approximately 20 tortoises that were found to be anorexic, chronically debilitated and with hepatic lipidosis. Ultrasonography demonstrated advanced non-progressive follicular development. At laparotomy up to 250 advanced follicles have been removed. Recovery in chronic cases has been prolonged compared to iguanas spayed because of similar follicular stasis. A guarded prognosis is best given where animals are likely to have been at reproductive standstill for several seasons. In such cases, keepers should be warned that a return to normal activity might take a long time, if it occurs at all. Juvenile animals, and those that have become anorexic following recent exposure to male animals in the current or prior season, tend to recover quickly from ovariectomy.

All cases spayed by the author have shown significant improvement over a period of nursing of 6–12 months. Oesophagostomy-tube feeding is occasionally necessary for as long as five months post surgery, but all spayed cases have improved and eaten the spring after surgery, having been prevented from hibernating the winter after spaying.

Medical treatment

Proligestone injection (Delvosteron®, Intervet 20 mg/kg) appears to induce ovarian regression in some individuals (McArthur 2000a). Because the response is not consistent in the limited number of animals that this author has treated, it is presumed that ovarian tissue is only responsive to progesterone at certain stages in the reproductive cycle. Cofactors may be necessary. Cases where positive subjective results have been observed (assessed using serial ultrasonography) have tended to be young or middle-aged animals. Older animals appeared to be poorly responsive, and showed little follicular regression following proligestone injections.

Caution should be exercised where significant hepatic compromise is likely because of lipidosis. Medical regression of follicles will result in transportation of ovarian material through the bloodstream to the liver, where it is processed. If the liver is unable to deal with this, then further compromise is likely. Blood values of albumin and calcium will remain elevated for a considerable time, and the clinician should not expect a return of appetite or activity until a significant amount of ovarian material has been metabolised. This may take several months.

Thyroid hormone appears to be necessary for normal ovarian regression in birds (Millam 1997) and supplementation in thyroidectomised birds induced photorefractoriness and gonadal regression. Thyroid supplementation may therefore also be indicated in chelonians.

Tamoxifen and gonadotropins may have therapeutic and/or preventative effects, but these have not yet been adequately investigated.

Induce ovulation

Early cases may respond to appropriate male contact (e.g. mating, pheromones, pre-mating biting and butting), if degenerate changes to the ovaries are still reversible. It is unknown when and how a meeting with a male tortoise, or a mating, will stabilise female reproduction. Personal observations suggest that females that are mixed with appropriate males every two to three years may maintain normal reproductive function better than those denied such contact. It is unknown how ovulation cues can be artificially replicated.

Prevention

- Maintaining chelonians in similar-species groups and allowing mating to occur every four years or so may assist prevention. Discourage keepers from maintaining mature tortoises as isolated females.
- Proligestone may prove effective in juvenile animals prior to the condition becoming significantly chronic.
- Photoperiod and temperature management should be assessed and possibly formalised.
- Nutritional disorders should be managed.

FROST DAMAGE

(Figs 11.14 & 11.38)

Aetiology

- Hibernation through sub-zero temperatures. Several days at sub-zero temperatures may be required.
- Generally, affected animals have been hibernated in boxes in unheated outhouses.
- Frost damage is unlikely with 'fridge hibernation' as temperatures can be more effectively monitored and controlled using digital devices and remote temperature probes. This is the method practiced and favoured by the author (SM).

Clinical signs

- Clinical signs may be non-specific and may just amount to a failure to thrive in the post-hibernation period.
- Sight damage: animals may be blind and show some degree of intra-ocular haemorrhage or other pathology such as clouding of the lens.
- CNS damage: animals with significant brain damage may circle or may be particularly inactive.
- Limb necrosis: distal limbs may become swollen and necrotic.

History

- Animals poorly monitored and/or protected during hibernation.
- Periods of sub-zero weather will have persisted for five days or more.
- Digital temperature probes and other methods of monitoring temperature are generally absent during hibernation.

Diagnosis

- History and clinical findings.

Treatment

- Long term nursing in the hope of recovery. Many cases will show gradual improvement and acclimatisation to altered brain and eye function over the following years.
- Assisted feeding/hand feeding may be required for some time, and clients should be informed of this and be shown appropriate techniques. Initial oesophagostomy tube placement may be beneficial.
- Hospitalise and manage limb necrosis and debility.
- Recovery is less likely when there are multiple neurological abnormalities (sight and balance).
- Sight damage may respond to long-term nursing in conjunction with vitamin A supplementation (Lawton & Stoakes 1989).
- Consider euthanasia where animals are particularly severely affected and their recovery is likely to involve a prolonged period of suffering.

GOUT

(Figs 6.30, 6.33, 6.37, 8.17–8.17a, 11.39)

Aetiology

Gout is the deposition of uric acid and urate salts within visceral tissues. Visceral and articular gout are both relatively common in reptiles. Zwart (1992) found gout in almost 4% of chelonian post-mortem examinations, but Keymer (1978a & 1978b) did not find it at all in his survey of necropsy studies of 144 tortoises or 129 turtles.

Gout stems from hyperuricaemia, which itself arises from an increased production of uric acid and/or a decreased excretion of uric acid. Increased production of uric acid is caused by excessive access to protein, especially where a herbivorous species is regularly fed on animal protein with high levels of purine bases. Decreased excretion of uric acid may be caused by:

- reduced perfusion of renal tissue through hyperparathyroidism, nephrosis/nephrotoxic medications;
- dehydration and/or haemoconcentration disease or water deprivation resulting in increased plasma osmolarity and reduced ability of renal tissue to excrete uric acid.

Tissues predisposed to the deposition of urates include the articular joints, the pericardial sac, liver, renal cortex and spleen. Gout in other tissues, such as the brain, gonads or sub-gingival and oral mucosal sites is also common at our surgery and is reported in the literature (Frye 1991a).

Once gouty tophi are formed, they are not readily reabsorbed. Veterinary management should address predisposing causes, correct dietary errors, and focus on adequate hydration and fluid therapy to reduce hyperuricaemia. Cooper & Jackson (1981) point out that adequate water availability can prevent gout.

The prevalence of gout in terrestrial, semi-aquatic and marine chelonians may differ, presumably because of the differences in nitrogenous excretion products. Terrestrial species tend towards uricotelism, which is associated with a high incidence of gout, and aquatic species tend towards aminoureotelism, which is associated with a much lower incidence of gout.

Zwart (1992) suggests that urate compounds will crystallise if the blood level of uric acid reaches 1500 µmol/l. Gout may also occur in chelonians with uric acid levels well below 1500 µmol/l, due to factors triggering off the crystallisation of uric acid complexes. Some of these factors are: high plasma osmolarity, tissue damage or the presence of high concentrations of uric-acid reactive cations.

The causes of gouty deposits are often multifactorial. A reduced flow of glomerular filtrates through nephrons results in a decrease in overall excretion of urate salts. Hyperuricaemia results, and this in turn leads to precipitation of urate complex microcrystals within tissues. These are commonly known as 'tophi'.

Renal excretion of uric acid in the proximal tubules is active, and appears to continue in the absence of a glomerular filtrate when uricotelic chelonians are dehydrated and experiencing high plasma osmolarity. This predisposes to obstruction of renal tubules by gouty tophi and possibly traumatic rupture of glomeruli.

Because the bladder recycles fluid and allows active excretion of potassium and urate as a solid precipitate, gout can also occur as a result of saturation of the bladder contents with urate toxins during periods of dehydration.

A relationship between solubility index in reptiles and soft tissue mineralisation is given by Divers (1998a).

- Normal reptiles
  Ca (mmol/l) × PO4 (mmol/l) is normally less than 9
  (Ca (mg/dl) × PO4 (mg/dl) is normally less than 55)
- Reptiles predisposed to soft tissue mineralisation.

When the solubility index is between 9–12, (55–70) mineralisation of diseased tissue (e.g. kidneys) occurs. If the solubility index rises above 12 (>70) then healthy tissue may start to mineralise (Divers 1998a).

Dehydration

Long-term dehydration predisposes to gout (Jackson & Cooper 1981a; Scott 1992). A history of failing to drink and/or urinate for a significant period (we suggest a *maximum* of ten days) should give a strong suspicion of hyperuricaemia.

Renal compromise

Animals receiving nephrotoxic drugs, such as the aminoglycosides, may develop gout because of renal damage. These drugs should be avoided where possible.

Drugs

Caution is advised before injecting nephrotoxic drugs or cation urate-reactive salts into seriously dehydrated chelonians or into the hind limbs (may trigger crystallisation of uric acid salts).

Probenecid may predispose to renal gout in dehydrated uricotelic species (personal observation).

Local inflammation
Tophi may occur more readily in areas of inflammation.

Nutrition
Excessive access to protein, e.g. where an herbivorous species is regularly fed on animal protein with high levels of purine bases, will predispose to gout. There is circumstantial evidence to suggest an increased prevalence of gout where diets with excessive protein are being fed (Scott 1992; Mader 1996b), however Zwart (1992) disagrees with this.

It can be hypothesised that animals with long-standing vitamin A deficiencies may develop gout because of renal dysfunction due to metaplasia of the renal tubules with decreased excretion of urates. Excessive vitamin D may be a factor, as is the case in birds (Speer 1997).

Hyperuricaemia may occur because of catabolism of body protein during negative energy balance, especially in combination with dehydration.

Clinical signs

As gout has various predisposing conditions, including dehydration, localised infections and inflammation and inappropriate nutrition, there are a variety of possible presentations. Clinical signs are generally non-specific.

Swellings
Where gouty tophi have been deposited within joints and superficial structures, swellings may be present. Animals with joint or limb swellings should be investigated for the possibility of gout, using blood biochemistry assessments, radiography and joint aspirates. These swellings must be differentiated from swellings resulting from other aetiologies. Zwart (1992) suggests that arthritis, pseudogout, renal hyperparathyroidism and *Mycobacterium* infections are important differential diagnoses of gout.

Depression
Where gouty tophi have been deposited within the central nervous system, or visceral organs such as the heart and liver, clinical signs are likely to be non-specific. Animals may be depressed, inactive and may respond poorly to external stimuli.

Dehydration
Some dehydrated animals will show decreased urine output and increased urine specific gravity. Signs of advanced dehydration, including reduced skin elasticity and sunken eyes, may be present in particularly severe decompensated cases.

History

In view of the various aetiological predispositions mentioned earlier, the history is generally non-specific:

- Animals may have been deprived of appropriate water sources or humidity.
- Animals may have been injected with medications whilst dehydrated.
- Chronic dehydration, inappropriate diet and catabolism of body protein during negative energy balance (starvation) are all common findings. Animals are commonly in this state in the immediate post-hibernation period. Many animals mistakenly diagnosed as suffering from post-hibernation anorexia will in fact have been chronically starved and dehydrated, and will be hyperuricaemic and hyperkalaemic.

Diagnosis

Radiography
Radiography can occasionally provide evidence suggestive of gout:

- Urate crystals are radiolucent and only radio-opaque when mineralised.
- Sodium urate is radiolucent, so may go unnoticed.
- Calcium urate is radio-opaque and easily demonstrated.

Ultrasound
Ultrasound scanning may demonstrate echodense areas of crystallisation within organs such as the heart and kidneys.

CT/MRI scanning
CT and MRI scanning may reveal tophi within the central nervous system.

Histopathology
Tissue histopathology is occasionally suggestive of gout.

Cytology
Cytology of aspirated material from tissue affected by gout will usually demonstrate inflammatory cells and urate crystals (Mautino & Page 1993; Speer 1997).

The Murexide test differentiates urates from calcium deposits. A single drop of nitric acid is mixed with the crystals on a slide, the slide is slowly flame dried, and one drop of ammonia is then added. If a red/purple colour appears, urates are present (Speer 1997).

Blood uric acid levels
Blood uric acid level measurements are unreliable for indicating the presence of gouty tophi. Often levels may have normalised by the time an animal is presented, while tophi created during periods of hyperuricaemia are still present.

Uric acid levels forcing active mineralisation may be in the region of 1500 µmol/l (Zwart 1992).

Solubility index may also be important in dehydrated cases (see renal disease).

Lower uric acid levels may still cause mineralisation, especially in the presence of localised inflammation.

Levels above 2000 µmol/l appear consistently fatal in terrestrial chelonians.

Treatment

Management of gout requires the removal of any predisposing causes. A specific protocol for the management of hyperuricaemia, hyperkalaemia and anuria for captive *Testudo* spp. in the post-hibernation period is given in the Hospitalisation section of this book.

Fluid therapy and forced diuresis

Fluids for maintenance should be regularly administered to all debilitated chelonians, unless the kidneys have become end-stage, the case is untreatable and euthanasia is to be performed.

Active fluid therapy is always advised in the management of recoverable hyperuricaemia. It counteracts any dehydration and allows injections of medications to be undertaken with a decreased risk of exacerbating further gout in animals with a high solubility index.

In acute conditions, intraosseous or intravenous fluids can be given using a syringe driver or paediatric (burette) giving set. In chronic disease, the oral and cloacal routes, using stomach tubes, oesophagostomy tubes, the coelomic and epicoelomic routes, bathing, and possibly Foley catheterisation of the bladder are ideal.

Most cases of gout can be considered to be the product of chronic disease. In these cases, this author would advise water at 1%–2% body weight per day orally, to induce regular diuresis. Thereafter 0.5%–1% is suitable for maintenance once regular urine output is established (e.g. > every third day).

Oral fluid therapy with hypotonic fluids (water) reduces plasma osmolarity to a level where glomerular filtration increases. In cases of dehydration this restores renal function, decreases blood uric acid levels and may partially dissolve uric acid precipitates.

Diet

Diets restricted in purines and protein should be used. During the initial stabilisation of a catabolic, anorexic reptile with signs consistent with renal failure, active administration of high-energy diets and fluids should be attempted. The use of an oesophagostomy tube or stomach tube should be considered. At our surgery, high-energy products such as Critical Care Formula® (Vetark, UK) are used in combination with fluids. As the patient begins to respond to treatment, it is gradually weaned on to its normal diet, in liquidised form, in combination with the opportunity to self-feed.

Medical treatment

Allopurinol

Allopurinol, a urease inhibitor, is commonly used in hyperuricaemic reptiles (Alluline®, Steinhard Ltd, 50 mg/kg orally daily), to reduce uric acid production. A dose commonly used by clinicians is 15–20 mg/kg. However, Kölle (2001) suggested that 50 mg/kg was more effective in *Testudo* spp.

There is limited information regarding its efficacy and possible long-term side effects in specific species. However, this author has used it without obvious adverse reactions for over 24 months in a variety of debilitated chelonians with recurrent or persistent hyperuricaemia. At our clinic we administer the drug by dissolving a 100 mg tablet in 5 ml of water and then offering 1–2.5 ml/kg daily in divided doses orally, or by stomach tube.

The duration of any treatment is dependent on the aetiology of the gout. If the cause of any hyperuricaemia is alleviated, then xanthine oxidase inhibitors may no longer be required. In post-hibernation hyperuricaemia, allopurinol therapy often continues for six or more months. According to Kölle (2001) renal deposits of urate take two to four months to dissolve when fluids (1% body weight per day) and allopurinol at 50 mg/kg are administered.

Historically, the literature gives various empirically derived dose regimes: 9.93 mg/kg was used by Figures (1997) for induction and 3.31 mg/kg was given orally every 24 hr for maintenance. Allometric scaling was used to derive the dose. Jackson & Cooper (1981a) suggest 15 mg/kg. Scott (1992) also suggests 15–20 mg/kg and 20 mg/kg was used by Wright (1992) and Martinez-Silvestre (1997).

Monitor blood uric acid and potassium levels throughout treatment.

Probenecid

Avoid medications such as this, which are aimed at increasing the renal excretion of uric acid, at least until effective flow of glomerular filtrate through renal tubules is considered likely, and especially in predominantly uricotelic species.

Manage and remove concurrent infection

It is particularly important to treat concurrent infection where tissue inflammation is present as a result. Specific treatment depends upon the organism involved, which should be identified. Diagnosis may involve biopsy, cytology, histopathology and microbiology.

NSAIDs

In humans, articular gout is often painful. However, caution should be taken when giving chelonians non-steroidal anti-inflammatory drugs (such as carprofen) in cases where hepatic compromise, renal compromise and dehydration may be present.

Surgery

Surgery may be indicated where uric acid deposits are compromising joints. Occasionally, this author (SM) has encountered masses that partially occlude the pharynx, necessitating debridement.

Surgical removal of urate salts from joint spaces of juvenile chelonians is described by Mader (1996).

Bladder urolithiasis may require surgical removal in gravid females presented with dystocia. Juvenile hatchlings are commonly presented with large deposits of what appears to be calcium or ammonium urate in the bladder, often putty-like. In one such case lithotrypsy was attempted, but without success (Calvert 1998: personal communication).

At our surgery we have attempted stone dissolution by bladder irrigation, and cloacal removal. Plastral coeliotomy is indicated if the animal is distressed with repeated straining to urinate (stranguria), and increased frequency of urination (pollakiuria).

Euthanasia

Consider euthanasia if renal function cannot be restored and fluid therapy is unable to restore renal output, or blood levels of uric acid remain above 2000 µmol/l or a persistent hyperkalaemia above 9 mmol/l is confirmed over time.

HEAT DAMAGE

(Figs 11.25–11.26, 11.68)

Aetiology

Care should be taken to identify the heat, light and humidity requirements and tolerances of species under examination.

Heat is a common cause of damage to chelonians:

- Plastron damage and infection may occur as a result of heating an immobile, faecally contaminated animal from below. Heat is best provided to basking species from above.
- Posterior paresis may result from excessive heating of the dorsal carapace.
- Excessive heat may lower the humidity for tropical species, inducing inactivity, anorexia, and dehydration.
- Often heat damage is the consequence of confining animals in a manner rendering them unable to escape from a heat source.
- Many reptiles appear to be unable to perceive a heat source to be excessive or unsuitable.
- Inactive, debilitated specimens under basking lamps are particularly vulnerable.

Clinical signs

Animals may be distressed, and with a profound orooculonasal discharge, if hyperthermic.

Areas of carapace and plastron necrosis and thermal trauma may be apparent, either immediately or over time.

History

- Confinement with an inappropriate heat source.

Diagnosis

- History and clinical signs.

Treatment

- Bathing in cool water.
- Cooled oral or parenteral fluids.
- Analgesia, antimicrobials, and wound care should be considered.

HEPATIC DISEASE

(Figs 6.34–6.35, 8.73–8.79)

Aetiology

- Infectious agents (viral, bacterial, fungal, protozoan or a consequence of metazoan migration).
- Inflammatory disease.
- Hepatotoxins.
- Nutritional disease.
- Neoplasia.
- Lipidosis.

Clinical signs

- Weight loss or gain.
- Anorexia.
- Biliverdinuria.
- Inactivity, lethargy and jaundice are typical of hepatopathy.

History

History will vary depending upon the aetiology.

Diagnosis

Blood biochemistry and haematology

As described in hepatic lipidosis:

- interpretation of results remains speculative;
- liver function tests are presently poorly defined;
- biliverdin assays may be useful (contact human hospitals).

Diagnostic imaging

- radiography;
- ultrasonography;
- endoscopy.

Cytology

Cytology of coelomic fluid may reveal inflammatory cells.

Biopsy and histopathology

Histopathology of liver tissue obtained at biopsy or post-mortem is the best method of assessing liver pathology. Samples may be obtained by biopsy endoscopically, during coeliotomy, or using ultrasound-guided techniques. Biopsy material should be treated appropriately for microbiological culture, histopathology or virology (potentially all three).

Post-mortem examination

Post-mortem examination may reveal abnormal size, colour, and texture of liver. Material should be submitted for further examination as described for biopsy, if funds allow.

Treatment

Depends upon the aetiology of the hepatopathy.

General

- Fluid and nutritional support are central to the management of all hepatopathies.
- All animals should have their environmental provision optimised.
- Viral hepatitis and disease considered potentially infectious should be barrier nursed.
- Lactulose® may help reduce encephalopathy.

Antimicrobials and antivirals

Bacterial and mycotic infections will benefit from antimicrobials as determined by clinical pathology investigations such as culture and sensitivity. Some animals may benefit from antivirals.

HEPATIC LIPIDOSIS

(Figs 6.35, 8.73–8.79)

Aetiology

Hepatic lipidosis is relatively common in captive chelonians (Bone 1992; Frye 1991b; Jackson 1991; Divers & Cooper 2000; SM: personal observation). Lipidosis would appear to be a normal physiological phenomenon related to provision of sustenance during hibernation and facilitating appropriate metabolism during vitellogenesis. Mounting evidence suggests it may also occur pathologically, when inappropriate husbandry and nutritional

problems unbalance homeostatic mechanisms. The literature suggests various predisposing factors, and the condition is likely to be multifactorial.

Inappropriate foods have been associated with obesity and hepatic lipidosis by authors such as Bone (1992). Bone suggests that overfeeding should be avoided, and that foods such as cat food, dog food, milk, jam, bread, potato chips and cheese should never be fed to herbivorous species.

Chronic hyperparathyroidism may also result in derangement of normal lipid metabolism (Akmal *et al.* 1990). There are potential links between hyperparathyroidism and deranged lipid metabolism and hyperparathyroidism may be a predisposing factor in the aetiology of some cases of hepatic lipidosis.

Finlayson & Woods (1977) describe the rupture of the aorto-pulmonary trunk of a Spanish turtle (*Mauremys caspica leprosa*) associated with thickened aneurysmal arteries where both lipid deposition and metastatic calcification were present.

Toxic iatrogenic lipidosis was associated with inappropriate injection of ivermectin (Bush & Teare 1983), however a cause-and-effect relationship is unproven.

Hepatic lipidosis is a relative phenomenon that must be interpreted in relation to season and possible primary disease, and quantified against the degree of fat deposition that can be accepted as being normal for the species, sex, and season. Post-mortem examination of relatively healthy animals that die as the result of trauma, revealed lipid deposition within the liver. It may be that this is a normal product of routine lipid storage, as liver is a major chelonian fat body.

Hepatic lipidosis rarely exists in isolation and goes hand in hand with a large number of chronic disease conditions described below. In many situations it may just be a clinical sign, as opposed to a primary disease in its own right.

Predisposing causes of inappropriate hepatic lipid deposition include:

- chronic hyperoestrogenism (e.g. follicular stasis);
- abnormal thyroid activity;
- diabetes mellitus;
- unsuitable diet containing excessive energy sources;
- chronic nutrient-deficient diet;
- influence of bacterial and mycotic toxins disrupting lipid metabolism;
- starvation;
- unsuitably long hibernation periods;
- unsuitable hibernation temperature;
- anorexia as the result of primary disease processes such as viral disease;
- excessive levels of parathyroid hormone (PTH) as a result of inadequate vitamin D production or a calcium-deficient diet;
- inadequate photoperiod or other exogenous factor stimulating inappropriate pre-hibernation metabolism and lipid storage.

Some specific factors

Natural photoperiod and temperature cues

Natural pre-hibernation metabolic changes and storage of energy (Jackson 1980) may cause benign hepatic lipidosis.

Exposure to inappropriate light and temperature cues

Hepatic lipidosis may result from inappropriate exposure to hibernation cues at an unsuitable stage in the season (likely to be common in animals maintained in the United Kingdom without supplementary heat and light).

Hyperparathyroidism

Hyperparathyroidism is associated with derangements in lipid metabolism (Akmal *et al.* 1990). This, or similar light-sensitive endocrinopathies, may be part of the natural cues triggering pre-hibernation fat deposition.

Inappropriate diet and dietary deficiencies

Excessive levels of dietary fat (Bone 1992) and relative amino acid and protein deficiencies (Frye 1991b) have been implicated in the aetiology of hepatic lipidosis in reptilian (Frye 1991b) and non-reptilian species (Johnson 1995).

Starvation

It has been suggested that there may be an inability of the liver to metabolise and process fat reserves mobilised during periods of starvation if some essential nutrients, such as lipotropic factors, are deficient (Frye 1991b; Johnson 1995; Hoefer 1997).

Clinical signs

Clinical signs are relatively non-specific:

- weight may be excessive, normal or decreased for the species and sex of animal;
- malaise;
- inactivity;
- anorexia;
- debility;
- concurrent disease likely.

History

The degree to which it is normal for captive chelonians to possess hepatic lipid deposition at different times of the year and stages of reproductive cycling is still poorly defined. The history will vary. It is usually relatively non-specific. Hepatic lipidosis can be expected to be concurrent with chronic disease in any captive chelonian, whatever the cause.

Often the animal has received poor husbandry and nutrition. There may have been a long period of inactivity and inappetence. Mature females suffering from follicular stasis and associated hepatic lipidosis may have been isolated from male contact and have failed to lay eggs for more than five years.

Diagnosis

History

Will vary depending upon cause and concurrent disease. Signs are typical of any chronic disease process. Divers & Cooper (2000) suggest they may include:

- reduced appetite, activity, fecundity and fertility;
- weight loss;
- complications following hibernation;
- altered faecal character and colour;

Blood analysis

Haematological changes are those common in non-specific chronic illness. These may be the product of concurrent disease.

Biochemical changes are also non-specific, and may involve alterations related to other primary or concurrent disease. No liver function test has yet been validated in a chelonian. No enzymes have been specifically pinpointed as having a high liver specificity, and no assay is currently available for biliverdin, the main chelonian bile pigment. No doubt this situation will improve with continued research.

Ultrasonography

Ultrasonography reveals liver size, shape and echogenicity. The lipidotic liver is reported to be hyperechoic (Center *et al.* 1993; Redrobe 1997; Divers & Cooper 2000).

Ultrasound may also facilitate guided biopsy, although likely to be somewhat crude.

Endoscopy

The liver is a major organ and is highly visible using prefemoral coelioscopy. Visual inspection often reveals a structure that is a pale yellow colour with friable, fatty texture. Visual inspection gives a strong indication of the condition of the liver. Rigid endoscopy allows biopsy, although samples are small.

Liver biopsy/post-mortem examination (PME)

Biopsy may be obtained endoscopically, during coeliotomy, or through ultrasound-guided biopsy. In hepatic lipidosis, the liver is pale and remarkably light in colour. The liver shape may give the impression that it is inflated and relatively full. During post-mortem examination it is important to compare the size and quality with other fat bodies.

Histopathology

Seven considerations relating to histopathological interpretation of liver tissue in relation to pathological lipidosis are outlined by Divers & Cooper (2000). These involve quantifying fat by area and by cell type. The determination of what is normal for the sex, species, age and season still remains somewhat difficult, but will ease as our database of presumed normal and lipidotic values increases.

Treatment

Treatment is often prolonged but even severely debilitated animals may improve given many months of care. Treatment is based on supportive measures and correction of predisposing factors (endogenous and environmental problems).

Treat any primary disease

Specific measures should be employed where concurrent disease, such as follicular stasis or stomatitis, is present.

Correct any husbandry inadequacies, especially those relating to malnutrition, random, uniform or uncontrolled day length, poor annual temperature regulation and poorly-regulated calcium metabolism.

Nutritional support

Most cases with severe hepatic lipidosis are anorexic and require extensive nutritional support. This may involve rehydration, electrolyte replacement and tube feeding with carbohydrate-rich products such as Critical Care Formula® (Vetark, UK). Amino acid supplementation may be indicated.

Empirical treatments

Historically, empirical treatments have included thyroid hormone supplementation (e.g. levothyroxine, Soloxine®, Arnolds, 22 µg/kg/day), with or without concurrent anabolic steroid injections (e.g. nandrolone, Nandoral®, Intervet, 1 mg/kg/week).

HYPERVITAMINOSIS A

(Figs 11.35–11.36)

Aetiology

The cause of hypervitaminosis A is overdosing by clinicians. Excess vitamin A causes degenerative changes in epithelia. Deficiency and excess can be confusingly similar. In clinical situations this can encourage inexperienced clinicians to administer further vitamin A parenterally when they have already reached adequate therapeutic levels. Such toxicity often results in horrific and frequently fatal degenerative epithelial pathology, especially of the skin.

Vitamin A is toxic at high levels in mammals and reptiles (Jensen & With 1939; Jarrett 1980; Frye 1989). Toxicity in chelonians manifests primarily as skin sloughing (Palmer *et al.* 1984; Zwart 1987; Frye 1991b; Boyer 1996b) and treatment of undiagnosed and non-specific debility in chelonians with vitamin A by injection is associated with a high risk of iatrogenic hypervitaminosis A (Palmer *et al.* 1984; Frye 1989). Therefore, vitamin A therapy by injection should be administered with great caution and only to animals with convincing evidence of deficiency. It is also advisable to bear in mind that water-soluble forms are more likely to result in toxicity than oil based (Palmer *et al.* 1984).

Most vitamin A injections available to veterinary practitioners are designed for large mammals. They are highly concentrated, making accurate dosing of small reptilian species virtually impossible (Boyer 1996b). Doses of vitamin A advised for the treatment of hypovitaminosis A show a wide degree of empirical variation.

Aquatic species are more likely to develop hypovitaminosis A than tortoises that are feeding on a balanced mixture of greens or foraging. Keep this in mind before giving vitamin A injections! Repeated doses should not be required if an inappropriate diet has been corrected (Zwart 1987).

Clinical signs

Toxicity in chelonians manifests itself primarily as skin sloughing (Palmer *et al.* 1984; Zwart 1987; Frye 1991; Boyer 1996b). Histopathologically it is characterised by acanthosis, proliferation of *stratum germinativum* (hyperplasia), and *parakeratosis* (Palmer *et al.* 1984; Frye 1991b; Boyer 1996b).

The classic clinical signs of hypervitaminosis A are epidermal detachment, often described as sloughing of the 'T-shirt and shorts'. The softer skin of proximal limbs generally blisters early in the course of the disease, revealing a moist, exposed dermis.

Changes to internal organs may also occur.

History

Excessive parenteral vitamin A will have been given, often repeatedly.

At this author's clinic (SM), many referred cases that appear unlikely to be vitamin A deficient have already received a vitamin A injection by the referring veterinarian.

Table 13.1 Levels of liver vitamin A following injection of vitamin A overdose (from Palmer *et al.* 1984)

	Water miscible vitamin A	Oil soluble vitamin A	Liver levels of vitamin A (wet wt)
Liver levels of vitamin A (wet wt) (10 days post injection)	2100–2850 IU/g (n = 3) dosed at 400,000 IU/kg 665–905 IU/g (n = 3) dosed at 100,000 IU/kg	255–1020 IU/g (n = 6) dosed at 400,000 IU/kg	10–80 IU/g (n = 3) untreated tortoises

Diagnosis

History

The administration of large doses of parenteral vitamin A is the usual cause of hypervitaminosis A. Treatment of undiagnosed and non-specific debility in chelonians with vitamin A by injection predisposes to iatrogenic hypervitaminosis A (Palmer *et al.* 1984; Frye 1989). Vitamin A therapy by injection should be administered with great caution and only to animals with clearly demonstrated deficiency. Repeated doses should not be required if an inappropriate diet has been corrected (Zwart 1987).

Clinical signs

As described previously.

Liver vitamin A assays

Palmer *et al.* (1984) measured the liver levels of vitamin A resulting after injection of *Testudo hermanni* with overdoses of oil-based and water-based vitamin A preparations. 400,000 IU/kg and 100,000 IU/kg were administered (see Table 13.1).

All animals receiving vitamin A in water-soluble form, at either dosage, developed severe epidermal sloughing. None dosed with oil-soluble vitamin A at 400,000 IU/kg had developed skin signs within 10 days of injection.

Treatment

Boyer (1996b) describes clinical recovery in cases of hypervitaminosis A displaying signs of skin sloughing as taking four to six months. Epidermal sloughing may require management for several months, in a fashion similar to the treatment of full thickness burns.

Further parenteral vitamin A administration must be discontinued. Supportive care includes fluid therapy and nutritional support.

Severely debilitated animals (often hatchling *Testudo* spp. and *Geochelone sulcata* at this author's clinic) may require feeding via oesophagostomy tube for up to six weeks. They are often too severely affected to maintain self-nutrition.

Lesion care

Systemic and topical antibiotic and antifungal preparations may be used. Most lesions benefit from regular cleaning with dilute skin disinfectants (Hibiscrub®; Pevidine®). Topical antibiotic and antifungal preparations can be applied as necessary (e.g. Flamazine®).

Temporary protection of lesions with petroleum jelly (Vaseline®, Cheseborough Ponds Ltd., London) during the acute stages of diseases helps to reduce fluid loss and wound contamination.

Analgesic medication should be administered, especially early in the course of treatment. Appropriate drugs are listed later in the Anaesthesia and Analgesia section of this book.

Severely affected cases may require euthanasia.

HYPOTHYROIDISM/HYPOIODINISM

Aetiology

Primary hypothyroidism in chelonians has not been conclusively described, although Norton *et al.* (1989) discussed this as a possibility in one case in a Galapagos tortoise (*Chelonoidis nigra*).

Animals ingesting diets low in iodine or high in goitrogens may have thyroid pathology and clinical signs, including goitre, compatible with hypothyroidism (Frye & Dutra 1974). Thiocyanates present in cruciferous vegetables such as mustard greens, collard greens, cabbage, bok choy, broccoli, Brussels sprouts, cauliflower, kale, mustard seed, rapeseed and turnips are proposed to be goitrogenic, and may be capable of inducing secondary nutritional hypothyroidism in extreme situations, especially in Galapagos tortoises (Innis 1994; Donoghue 1996).

In the literature, Galapagos tortoises (*Chelonoidis nigra*) and Aldabran tortoises (*Dipsochelys elephanina*) are often described as susceptible to iodine deficiency and the effects of goitrogens, but this suggestion is anecdotal.

The true prevalence of hypothyroidism is unknown. 'Sick euthyroid' syndrome may be common: animals with non-thyroid illness have been found to have low or undetectable thyroid hormone levels (Raiti & Haramati 1997).

Clinical signs

Clinical signs are non-specific, as hepatic lipidosis:

- goitre;
- malaise/debility;
- inactivity/decreased activity;
- anorexia/decreased appetite;
- weight gain/obesity.

The role of thyroid hormones in the mediation of female reproduction also appears to be unknown, but influences are possible.

Subclinical disease is likely and may be common.

History

History may also be non-specific, as hepatic lipidosis:

- diet rich in goitrogens;
- gradual deterioration;
- potentially decreased fertility.

Diagnosis

History, histopathology of thyroid tissue, potentially blood levels of thyroid hormones (but sick euthyroid syndrome may be common).

Very little has been published about thyroid hormone measurements in chelonians, and no information is available on dynamic thyroid function tests:

- Healthy adult Galapagos tortoises (n = 3) had T4 levels between 1.08–1.54 ng/dl and T3 levels 33–93 ng/dl (Norton *et al.* 1989). A tortoise proposed as deficient had a T4 level of 0.29 ng/dl and a T3 level of 4.50 ng/dl.
- T4 Values in *Testudo* spp. were 10–25 nmol/l in 12 healthy animals in September (Harcourt-Brown 1998).
- Normal T3 and T4 values are also available for the eastern painted turtle (*Chrysemys picta*) (Sawin *et al.* 1981) and the green sea turtle (*Chelonia mydas*) (Licht *et al.* 1985).

Treatment

Better prevented by dietary correction and iodine supplementation.

Iodine supplementation

Donoghue (1996) points out that safe levels of dietary iodine for tortoises are unknown, but suggests a quarter to a third of mammalian levels (0.3 mcg/kg body weight). Toxicity levels are also unknown, but Donoghue (1996) suggests iodine toxicity could be possible with significant over-supplementation.

Thyroid supplementation

Thyroid supplementation (e.g. levothyroxine, Soloxine®, Arnolds). An empirical dose is 22 mcg/kg/day). Norton *et al.* (1989) also found a good response to administration of a synthetic levothyroxine in a Galapagos tortoise diagnosed as being hypothyroid.

HYPOVITAMINOSIS A

Aetiology

Vitamin A has a major role in the production and maintenance of healthy epithelial surfaces. It also has important roles in structures concerned with vision.

There are varying opinions in the literature regarding how likely it is for chelonians to be vitamin A deficient, as levels are generally good within most chelonian diets, especially those of herbivores. Foods rich in β-carotene, the plant precursor to vitamin A, include dark leafy greens (spinach, dandelions, turnip and mustard greens, bok choy and broccoli, as well as yellow/orange-coloured fruits and vegetable such as squash and carrots) (Boyer 1996b). Deficiency in herbivorous tortoises is possible where foraging is prevented and a diet restricted to components low in β-carotene, such as iceberg lettuce and cucumber, is offered.

Because of its important roles in maintaining normal epithelial structure and in sight, many husbandry texts and advice sheets suggest that deficiency is relatively common, and that chelonians require a constant low-level intake of vitamin A in order to avoid deficiencies. However, chelonians fed a balanced and varied diet containing adequate amounts of vitamin A are unlikely to benefit from such supplementation. It is unlikely that oral supplementation will result in hypervitaminosis A, but great care should be taken to avoid unnecessary parenteral administration of vitamin A, because toxicity is easily caused.

In Mediterranean and other terrestrial tortoises vitamin A deficiency is only likely to be a problem if a deficient diet has been fed for some considerable time, or tortoises have been anorexic for long periods. Reptiles presenting with signs of vitamin A deficiency are likely to be deficient in other nutrients as well (Fowler 1980). In such situations, constant supplementation with a low level of oral vitamin A is advisable. (Suitable products available in the United Kingdom include ACE High®, Vetark; Arkvits®, Vetark; BSP drops®, Vetark; Vionate®, Sherley's.)

Historically, hypovitaminosis A appears to have been relatively common in omnivorous/carnivorous chelonians, such as juvenile red-eared sliders (*Trachemys scripta elegans*), where it was first described by Elkan & Zwart (1967). Such animals are commonly fed on restricted diets in captivity and dietary deficiency can be further exacerbated where animals preferentially refuse some food components in their normal diet and become obsessed with others.

Frye (1989) suggests that the yolk remaining at the time of hatching in many reptiles usually furnishes requirements for around six months, and that deficiency can become dramatically apparent after this point, when stores will have become depleted.

In the United Kingdom a ritual pre-hibernation vitamin A injection has unfortunately become common practice for Mediterranean tortoises. We believe that such an injection fails to address any underlying nutritional problem and is of limited therapeutic value. If pre-hibernation tortoises are suspected of being deficient in vitamin A, it would be wiser to prevent hibernation and improve standards of nutrition. Injection with a vitamin A preparation at a time when the animal is becoming metabolically inactive would be imprudent and may risk toxicity.

Clinical signs

Deficiency of vitamin A results in squamous metaplasia and degeneration of epithelial surfaces such as conjunctiva, gingiva, pancreatic ducts, renal tubules, skin and lung alveoli (Elkan & Zwart 1967; Fowler 1980). Sub-clinical disease may be common in poorly maintained animals fed inappropriate diets.

As vitamin A deficiency results in changes to epithelia that are widely distributed throughout the body, a wide variety of clinical presentations are possible. Acute deficiency in semi-aquatic chelonians is generally associated with ocular signs. Chronic deficiency in terrestrial chelonians may result in changes in respiratory, hepatic, renal and pancreatic epithelia, and so may manifest as a generalised debility. Chronic vitamin A deficiency may predispose to post-hibernation blindness.

Signs of hypovitaminosis A include:

- blepharitis;
- conjunctivitis;
- rhinitis;
- lower respiratory tract disease;
- cutaneous abnormalities;
- general decline.

The chelonian may be seriously ill because of some other reason, such as viral disease, and also have clinical signs consistent with hypovitaminosis A.

Ocular signs

The classically-described presentation in young turtles involves oedema, inflammation and infection of the conjunctiva, resulting from squamous metaplasia of the Harderian glands. We have seen similar ocular lesions in a *Testudo* with liver vitamin A levels of 1.2 IU/g wet weight of liver. Chronic deficiency in terrestrial tortoises may predispose to sight impairment. Oral supplementation is advised in any tortoise with poor pupillary and menace reflexes.

Ocular signs associated with hypovitaminosis A include:

- ocular discharge (Boyer 1996b & c);
- blindness (Boyer 1996b & c);
- post-hibernation blindness (Lawton & Stoakes 1989);
- blepharoedema;
- dermal hyperkeratosis of the external surfaces of the eyelids of juvenile sea turtles (Frye 1991b).

Dermal signs

Little specific literature appears to link dysecdysis in terrestrial chelonians with hypovitaminosis A. Skin sloughing (Boyer 1996c), middle ear infections, and aural abscesses (Boyer 1996b) have been linked with hypovitaminosis A in chelonians.

Several cases presented at our surgery with chronic, non-specific nutritional disease apparently related to hypovitaminosis A showed dramatic dysecdysis that appeared to respond well to improved husbandry, nutrition and oral vitamin A supplementation.

Respiratory signs

Upper and lower respiratory tract infections linked to possible hypovitaminosis A are described by many authors (Fowler 1980; Rowley 1985; Frye 1991b; Jungle & Miller 1992; Voprsalek & Simunek 1994; Boyer 1996b & c). Both Fowler and Frye point out that hypovitaminosis A and respiratory disease in chelonians such as *Gopherus agassizii* share similar clinical signs. These include nasal discharge, oral discharge, depression, anorexia, weight loss, open-mouthed breathing and dyspnoea.

Rowley (1985) investigated an eastern box turtle (*Terrapene carolina*) with swollen eyelids and rhinitis associated with vitamin A deficiency. Rowley linked respiratory signs such as open-mouthed breathing, wheezing and a nasal discharge with degeneration of epithelial tissues.

Voprsalek & Simunek (1994) reviewed diseased reptiles presented to a Prague clinic over a long period and concluded that 1093 out of 1401 reptiles treated may have had some form of deficiency of vitamin A. Out of 989 turtles, mostly recently imported *Chrysemys*, 935 were considered to be vitamin A deficient. The authors suggested that pneumonia occurred as a complication in 31% of the turtles, especially if they had been kept at low temperatures.

Other systemic signs

Other systemic signs may be attributed to hypovitaminosis A or appear in animals diagnosed as concurrently deficient in vitamin A. These include:

- failure to lay eggs, especially box turtles (Boyer 1996b & c);
- hepatic lipidosis (Elkan & Zwart 1967);
- renal failure, inguinal and axillary swelling secondary to renal failure (Lawton 1989).

History

The history will usually include one or more of the following:

- inappropriate nutrition and husbandry;
- may appear 'suddenly' in juvenile animals that have been kept for around six months or more on an unsuitable diet, when yolk reserves become exhausted;
- may follow periods of anorexia following capture, relocation and captivity;
- semi-aquatic turtles, fed diets of lettuce and a restricted variety of meats (e.g. bacon only), are prone to vitamin A deficiency, especially juveniles after recent importation or relocation;
- terrestrial tortoises maintained on a long-term diet of iceberg lettuce and cucumbers, without vitamin supplementation or ability to forage, are also susceptible to vitamin A deficiency. According to Rosskopf *et al.* (1982) a lettuce-only diet has unsuitable calcium and phosphate levels, and is deficient in vitamin A and vitamin B complex.

Diagnosis

Diagnosis is usually based upon the consideration of a combination of factors. The differential diagnosis of suspected hypovitaminosis A includes any condition causing eye pathology, such as trauma to an eye, an ocular foreign body, irritation from airborne particles such as pollen or spores, and conjunctival infection. Any cause of anorexia in the post-hibernation period should also be included, together with any generalised illness and any condition causing upper respiratory tract disease, such as herpesvirus infection or mycoplasmosis.

All cases of unexplained anorexia and respiratory tract disease should undergo a thorough diagnostic work up, including examination of the eyes and visual reflexes.

Various factors can be used to construct the diagnosis of vitamin A deficiency including:

- dietary history;
- clinical signs;
- histopathology of tissue biopsies (squamous metaplasia of epithelial surfaces);
- vitamin A assays of liver or blood (currently quite complex and impractical).

Vitamin A assays

A definitive diagnosis of hypovitaminosis A can only be achieved by assaying vitamin A levels within a specific tissue such as blood or liver and comparing the value with species normal. In practice this is unrealistic if the amount of material required is in excess of that present within the animal. The amount of blood and liver that can be harvested without detriment by phlebotomy or biopsy is limited in the case of live specimens.

A lack of published normal chelonian values means that a diagnosis of hypovitaminosis A in practice must be subjective.

Liver biopsy and vitamin A assay

Endoscopic and other methods of liver biopsy are described elsewhere. The weight of liver requested for vitamin A assay in the United Kingdom often appears to be 1 g or more. This is hard to do in animals of less than 70 g. It may be necessary to sacrifice an

animal and assay its liver at necropsy in order to confirm a suspected diagnosis, making the assay of limited diagnostic value.

Palmer *et al.* (1984) found that three normal, untreated *Testudo hermanni* had liver levels of vitamin A of 10, 30 and 80 IU/g liver. Diseased animals may have vitamin A levels below 9–19 IU/g liver, with normal values in non-chelonians being over 1,000 IU/g (Elkan & Zwart 1967; Scott 1992). This author (SM) has recorded a liver value of 0.36 µg/g wet weight in a juvenile *Testudo marginata* with clinical signs consistent with hypovitaminosis A, including a caseous bilateral conjunctivitis, rhinitis, hepatitis and pneumonia (noted at post-mortem examination). Using a conversion factor of 3.36, this was equal to 1.2 IU/g.

Plasma retinol values

Lack of normal vitamin A levels limits the usefulness of this method of diagnosis. Mean plasma retinol values in eight male and eight female pancake tortoises were found to be 0.62 µg/dl and 0.32 µg/dl respectively (Raphael *et al.* 1994).

Treatment

As always, prevention of nutritional diseases such as hypovitaminosis A is better than cure. The vitamin A content of some chelonian diets has been listed earlier.

General management

Dietary management

The diet of any chelonian presented for examination should be assessed for provision of suitable amounts of vitamin A and other nutrients. Donoghue & Langenberg (1996) suggest 2000–10 000 IU/kg dietary dry matter for herbivorous tortoises. Fowler (1980) suggests a daily dietary allowance of vitamin A to be 210 IU/Kg body weight for terrestrial tortoises, however this level may be arbitrary.

Where deficiency is strongly suspected, the clinician can supplement with oral vitamin A products (examples of products available in the United Kingdom include ACE-High®, Vetark. UK; BSP drops®, Vetark, UK; Arkvits®, Vetark, UK; Vionate®, Sherley's). Omnivorous species can be fed small amounts of liver on a regular basis (e.g. weekly). Oral dosing with natural sources of carotene, preformed vitamin A, or both, is safer than the use of injections.

Management of lesions

Treatment of lesions should be in parallel with dietary and husbandry improvements.

Boyer (1996b) advises that cellular debris under the eyelids of semi-aquatic chelonians suffering from hypovitaminosis A should be carefully removed with a blunt probe. The subsequent administration of antibiotic ointments is optional (Figs 13.57–13.59).

Secondary conditions such as dysecdysis with secondary dermal infections should be treated.

Vitamin A injections

Iatrogenic hypervitaminosis A can occur even after a single vitamin A or multivitamin injection. Injections containing vitamin A

Fig. 13.57 Conjunctivitis and presence of caseous plaques associated with hypovitaminosis A in a red-eared slider (*Trachemys scripta*).

Fig. 13.58 With the animal sedated and local anaesthetic applied to the eye, or alternatively under general anaesthesia, caseous material is gently removed. Fine tissue forceps and dampened cotton buds can be used.

Fig. 13.59 The eye is then irrigated and an antibiotic preparation applied. Underlying problems with inappropriate nutrition should be corrected.

should be used with great caution, or possibly not at all. Oral dosing with natural sources of carotene, preformed Vitamin A, or both, is safer and is preferred.

In *Testudo hermanni*, acute hepatic uptake of vitamin A following parenteral injection was shown to be higher when water-miscible, as opposed to oil-based products, were used (Palmer *et al.* 1984). This may mean that only oil-based products should be used in chelonians, and that water-miscible products should be avoided. This may reduce the possibility of iatrogenic hypervitaminosis A.

There appears to be no standardised dose of parenteral vitamin A therapy in the literature. Doses of vitamin A for the treatment of hypovitaminosis A show a wide degree of empirical variation. Boyer (1996b) and Fowler (1980) suggest subcutaneous injections of 1500–2000 IU/kg once a week, for two to six weeks, whereas Donoghue & Langenberg (1996) suggest a single dose of 200–300 IU/kg body weight by injection. Palmer *et al.* (1984) even found doses as high as 100,000 IU/kg in their review of literature. These levels appear to exceed toxicity!

HYPOVITAMINOSIS B_1 (THIAMINE)

Jackson & Cooper (1981) suggest that diets containing high proportions of raw and/or frozen fish containing thiaminase are responsible for thiamine deficiency in water snakes, aquatic chelonians and crocodilians.

Clinical signs described are non-specific and centre on weight loss, however this diagnosis should be considered wherever neurological signs (e.g. swimming in circles) are present in association with a fish-based diet.

Treatment consists of alterations to the diet and supplementation with thiamine-containing products (e.g. BSP drops®, Vetark, UK).

Fig. 13.60 Dorsoventral survey radiography of a gravid red-eared slider (*Trachemys scripta elegans*) demonstrating significant metabolic bone disease and the presence of eight modestly calcified eggs.

This animal needs to be treated for nutritional hyperparathyroidism which has been exacerbated by increased calcium demands following ovulation.

Several stones are apparent within the digestive tract. These are likely to be in the stomach and/or large intestine. In this case they are incidental findings but they do suggest an inappropriate substrate and that the animal may have been inadequately fed.

LOWER DIGESTIVE TRACT DISEASE

(Upper digestive tract disease is discussed earlier under the headings stomatitis, rhinitis, and virus-associated diseases.)

Lower digestive tract (gastric and intestinal) diseases appear common in chelonians. Bacterial, fungal, and parasitic organisms are frequently suggested to be potential gastrointestinal pathogens. Upset of the normal gastrointestinal flora may lead to significant dysbiosis, with overgrowth of pathogens, poor digestion and production of fermentation toxins. Mechanical obstructions by foreign bodies, intussusception or intestinal volvulus may also occur. Neoplastic diseases of the gastrointestinal tract have occasionally been reported in chelonians.

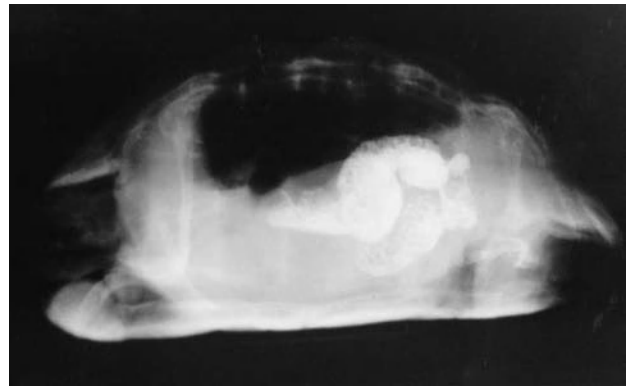

Fig. 13.61 Lateral radiograph of a leopard tortoise (*Geochelone pardalis*) with an obvious obstruction of its large intestine resulting from substrate ingestion. Here the obstruction has been revealed using a mixture of a human, water-soluble lubricant jelly and Iohexol (Omnipaque®, Nycomed) in a contrast radiographic study.

This type of impaction is likely to resolve with treatment as Fig. 13.63.

INTESTINAL IMPACTION/ OBSTRUCTION

(Figs 13.60–13.69, 8.43a–b, 8.44a–b, 8.45)

Aetiology

Gastrointestinal obstructions are commonly seen in captive and free-ranging chelonians. Marine turtles are often found debilitated by gastrointestinal obstruction by plastic bags. Freshwater aquatic turtles are often presented after ingestion of fishing gear. Captive tortoises, because of pica, may be presented with obstruction by gravel or other indigestible bedding materials, such as kitty litter or corncob. An increasing number of hatchling tortoises are becoming obstructed by crushed walnut shells sold as reptile bedding in the United States.

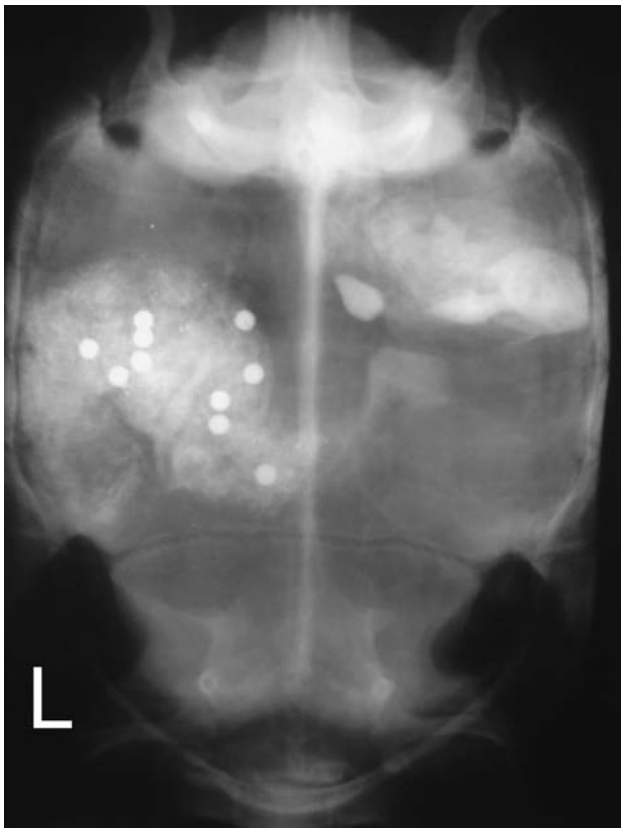

Fig. 13.62 *Testudo ibera*: intestinal obstruction caused by gastric neoplasia. There was a secondary gastroduodenal intussusception. This animal is undergoing a contrast study using BIPS.

Fig. 13.63 Dorsoventral radiograph of a North American box turtle (*Terrapene* sp.) with an obvious radio-opaque obstruction of the large intestine as a consequence of ingesting large amounts of yellow sand substrate in its enclosure.

This type of impaction generally resolves with improvements in hydration status, cloacal irrigation, lavage and lubrication and oral laxative administration.

The substrate should be changed to something more suitable. Surgery is rarely required.

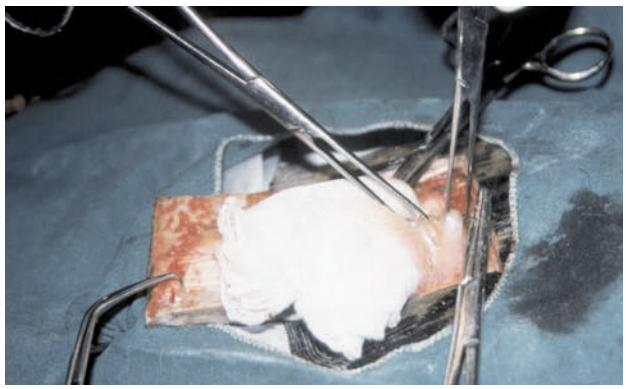

Fig. 13.64 Following plastron osteotomy, the visceral organs are identified and the obstructed digestive tract is exteriorised. The coelomic cavity is packed off before opening the digestive tract in order to reduce unnecessary contamination.

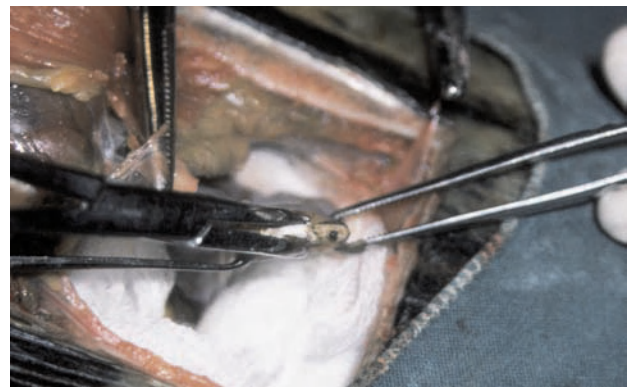

Fig. 13.65 Stay sutures and forceps can be used to stabilise and exteriorise intestines, allowing incision and location of foreign material.

Obstruction in the absence of foreign bodies has also been reported, and may be due to the neoplasia, intussusception or volvulus (Hasbun *et al.* 1998).

This author has observed intussusception and obstruction as a consequence of gastric neoplasia in *Testudo graeca*.

Not all foreign material present in the digestive tract is pathological. Small amounts of foreign material in the digestive tract are often incidental radiographic findings, especially where material is seen in the large intestine, and in the absence of disease. Semi-aquatic animals such as red-eared sliders seem especially predisposed to ingestion of non-obstructive foreign bodies, when housed on gravel substrate.

Terrestrial tortoises seem predisposed to intestinal impaction when housed on sand substrate (even with brands marketed as unlikely to result in impaction). The colour of the sand and the use of resins appear influential. *Testudo* species occasionally appear to ingest sands that are red or yellow, perhaps mistaking them for food.

This author (SM) would advise careful selection of substrate material during the hospitalisation of chelonians to avoid possible impaction. Suggestions are given in the General Care and Hospitalisation sections of this book.

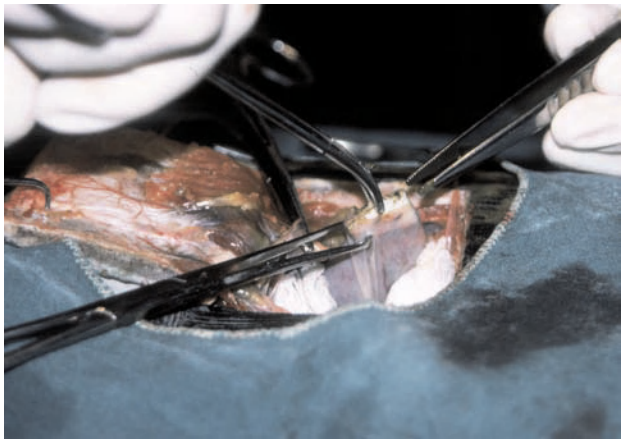

Fig. 13.66 Any contaminated instruments must be discarded and great care must be taken to limit contamination of the coelom with contents of the digestive tract.

Fig. 13.67 Here the digestive tract is quickly closed to produce an initial 'seal' retaining digestive tract material and preventing unwanted spillage. This area is then oversewn with a continuous suture to produce a watertight seal.

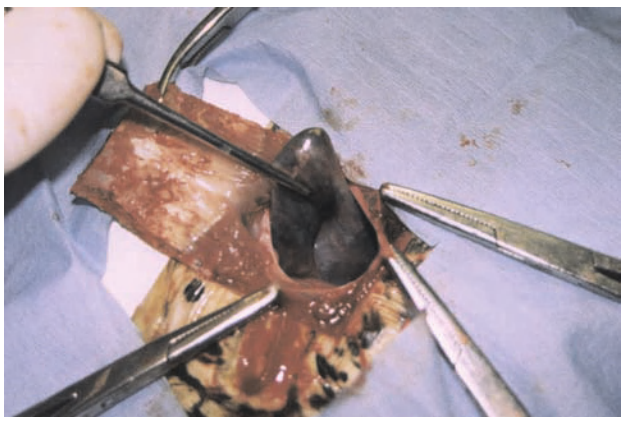

Fig. 13.68 Impactions of the large intestine are relatively easily exteriorised. Here the sheer volume of material within this animal's large intestine necessitates removal.

Fig. 13.69 Over 100 stones of this size (about 20 typical examples are illustrated) were removed from a 400 g juvenile leopard tortoise (*Geochelone pardalis*). The animal had become immobile, anorexic, weak and dyspnoeic, but recovered quickly following correction of fluid and electrolyte imbalances.

Clinical signs

Clinical signs of obstruction are non-specific and may include:

- anorexia and listlessness;
- straining (lifting the caudal plastron by extension of hind limbs);
- regurgitation of fluids and food, especially those given by gavage;
- cachexia and generalised debility will become more severe with chronicity;
- animals may show clinical signs of advanced dehydration (sunken eyes etc.) at presentation if the condition is chronic.

History

The history is usually non-specific and often relates to a period of general decline and anorexia.

Occasionally, animals will have been observed ingesting foreign material or substrate.

Husbandry may appear to have been generally of good standard, but animals may have been housed with an unsuitable substrate as described earlier.

Diagnosis

Diagnosis is generally made using imaging techniques. Often radiodense material or ileus gas patterns may be observed. Various techniques may all be helpful:

- plain and contrast radiography;
- ultrasonography;
- MRI scanning;
- endoscopy.

Dynamic imaging studies, BIPS, water-soluble gel (e.g. KY Jelly®) and contrast media, are described in further detail in the Diagnostic Imaging section of this book.

Treatment

- Remove unsuitable substrate.
- Management of intestinal foreign bodies and obstruction is similar to that in domestic mammalian species. In some cases, medical management may help small foreign bodies to be passed with faeces. However, large amounts of foreign material may need to be removed endoscopically or surgically.

Medical treatment

Medical management of foreign bodies should include systemic and oral rehydration. Bathing terrestrial tortoises in warm shallow water for 30–60 minutes often stimulates drinking and defecation, and may promote peristalsis.

Enemas may also be useful, and may be administered using warm water or mineral oil. The use of oral gastrointestinal lubricants, such as mineral oil, may also be attempted, but care must be taken to avoid aspiration. This author (CI) has also used feline laxatives (e.g. Laxatone®) and Lactulose syrup® orally in tortoises at standard mammalian doses, with good clinical results.

Intestinal motility agents such as cisapride, metoclopramide or erythromycin may be useful in some cases. Initial reports of their use, however, have not been encouraging. Tothill *et al.* (1999) found that metoclopramide, cisapride and erythromycin had no discernible effect on the gastrointestinal transit time of indigestible plastic markers in *Gopherus agassizii*. Hydration status, core temperature, nutritional status (especially with respect to calcium metabolism) and dietary fibre/moisture content would appear to exert a more significant influence.

These drugs deserve further investigation, and should be considered for clinical use in chelonians. As in domestic species, animals with suspected complete obstructions should not be treated with motility stimulants.

Surgical treatment

Ileus patterns that remain unchanged over 24 hours may warrant surgical intervention to avoid irreversible necrosis.

Surgical management of obstruction, intussusception or volvulus is generally through a coeliotomy, as discussed in the surgery section.

ENTERITIS AND COLITIS

Clinical significance

Enteritis and colitis are commonly seen in chelonians. Keymer (1978a) found enteritis and/or colitis in 27% of tortoise necropsies. These lesions are often associated with bacterial invasion of the intestinal wall. Organisms found in the lesions are generally components of the normal intestinal flora that have been allowed to invade the mucosa due to immunocompromise and malnutrition. Enteritis and colitis are commonly seen in newly imported specimens as well as specimens housed at low temperatures, hibernated inappropriately and fed inadequate diets.

Diagnosis

(see also Clinical Pathology)

Endoscopic evaluation of the digestive system may be required to obtain a definitive diagnosis. Culture of faeces, although generally not very rewarding due to the large numbers of normal enteric organisms, may be useful if a pure culture of a suspected pathogen is obtained.

Treatment

(see also Therapeutics)

- Systemic antibiotics effective against Gram negative and anaerobic organisms, as determined by culture and sensitivity, may be administered.
- Supportive environmental care and nutritional/fluid support should be offered.
- Probiotic agents may be of some benefit.

FUNGAL ENTERITIS

Clinical significance

A variety of fungal organisms may be found on cytological examination of faeces of ill tortoises. However, no specific mycotic organisms have been implicated as primary chelonian pathogens.

Fungal infection generally is promoted by high humidity, malnutrition, overcrowding and inadequate environmental hygiene (Jacobson 1994). The use of antibiotics may also promote fungal overgrowth.

Published reports of gastrointestinal fungal infections are rare, but fungal overgrowth appears to be relatively common in the clinical setting. Documented cases of gastrointestinal fungal infections were reviewed by Jacobson (1994) and include *Penicillium* infection of the stomach in *Chelonidis nigra*, *Basidiobolus ranarum* from the mouth of *C. gigantea*, and *Paecilomyces* from the mouth and stomach of *C. gigantea*. Zwart & Buitelaar (1980) describe yeast infection of the gastrointestinal tract of anorexic *Testudo graeca*, with successful treatment with oral nystatin.

Fungal organisms are frequently encountered associated with lesions of bacterial or viral stomatitis and upper digestive tract disease. Topical antifungal medications such as nystatin, miconazole, or silver sulfadiazene are often useful in treating these lesions, in combination with other medications.

Diagnosis

(see also Clinical Pathology)

It is not always simple to decide whether to treat gastrointestinal fungal infections. Certainly, if endoscopic biopsy reveals the presence of fungi with associated pathology, the decision is simplified. However, simply seeing fungal organisms on faecal or gastric-wash cytology may not necessarily indicate true infection. Generally, the judgement of the clinician must be used to decide if the number of organisms is high enough to justify treatment.

Treatment

(see also Therapeutics)

The choice of medication for treatment of gastrointestinal fungal infection depends partly on whether the infection is suspected to be affecting other parts of the body. Nystatin has been used commonly and appears to be safe in treating most gastrointestinal fungal infections, but is not absorbed systemically. For more widespread fungal infections, drugs such as ketoconazole, itraconazole, or fluconazole may be indicated. Amphotericin B has also been reportedly used in chelonians, but its potential toxicity must be considered. See Therapeutics for more detailed information on these medications.

It may be also be beneficial to attempt to restore a normal gastrointestinal flora and chemical milieu by tube feeding and use of probiotics. Inoculation of the gut with beneficial bacteria has not yet been proven effective in tortoises, but has been useful clinically. Prepared probiotic products containing *Lactobacillus* are now available commercially for reptiles and may be of use. Alternatively, faeces from a healthy conspecific may be tube fed.

AMOEBIASIS

Clinical significance

Amoeboid organisms are common in chelonian faeces. They have in the past been considered non-pathogenic for chelonians, but cases of pathology have certainly been reported and are increasing in frequency.

Chelonians may be a source of infection for other reptiles where pathogenicity is potentially greater. Intestinal biopsy associating organism and pathology, or histopathology of organism and pathology may go some way towards suggesting a pathological host/parasite relationship.

Jacobson *et al.* (1983) points out that *Entamoeba invadens* is not a natural pathogen of wild snakes and is generally present as a result of poor hygiene and mixing of reptile species. In snakes the organism is considered highly pathogenic, and therefore snakes and chelonians should be housed separately if possible.

Jacobson *et al.* (1983) describe an outbreak of disease in 200 out of 500 recently imported red-foot tortoises (*Geochelone carbonaria*). Clinical signs included anorexia, listlessness and watery diarrhoea leading to eventual death. At post-mortem, amoeboid organisms were present in hepatic sinusoids and necrotising hepatitis may have resulted from spread from the intestines via the bile duct (Jacobson 1986).

Pathogenicity has also been reported in leopard tortoises *Geochelone pardalis* (Jacobson *et al.* 1983; Jacobson 1994), where disease was characterised by anorexia, malaise, diarrhoea and high mortality. Sporadic single cases are described in other chelonians (Keymer 1981).

Amoeboid protozoans causing an enterohepatitis have fatally infected captive green and loggerhead sea turtles. As amoeboid organisms cannot live in salt water it was assumed that the source was infected food. Control is probably unnecessary in salt-water facilities, but hygienic food preparation should be ensured (George 1997).

Giant species of chelonians are suggested to be especially susceptible (Klingenberg 1993).

Amoebae are commonly seen in the faeces of many southeast Asian turtles imported into the United States (*Cuora* spp., *Cyclemys* spp., *Geoemyda* spp., *Pyxidea mouhoutii*). Eliminating these organisms appears to improve the overall survival rate of these imports.

Diagnosis

(see also Clinical Pathology)

Faecal examination

This is the usual method of diagnosis, with cysts and trophozoites being indicators of infection (Jacobson 1983). Culture techniques and repeated sampling may be required to identify intermittent shedders and carriers (Denver *et al.* 1999). In the infection described by Jacobson *et al.* (1983) no amoebae were detected in faeces.

Histopathology

According to Jacobson *et al.* (1983), histopathology of duodenum and liver were diagnostic in an epizootic of amoebiasis in *Geochelone carbonaria* where no organisms or cysts were apparent by faecal microscopy.

Culture

Repeated sampling may be required to identify intermittent shedders (Denver *et al.* 1999).

ELISA

Newer diagnostic modalities, such as ELISA testing, may increase the sensitivity of amoeba detection.

Treatment

(see also Therapeutics)

The choice of medication for treating amoebiasis in humans often depends on the suspected location of the infection and the severity of the infection. Drugs are generally classified by their site of action and their ability to kill trophozoites and/or cysts. If a patient is an asymptomatic cyst shedder, a drug that is active within the intestine and effective against cysts is chosen. If the patient suffers from extra-intestinal infection, combination therapy is generally used to kill migrating trophozoites as well as intestinal cysts (Garcia & Bruckner 1997).

Amoebicidal drugs

Metronidazole

The most commonly reported therapy for reptilian amoebiasis has been metronidazole, given orally at relatively high doses and relatively infrequent intervals (Stein 1996). Recent pharmacokinetic studies in the green iguana and the yellow rat snake have shown that a metronidazole dose of 20 mg/kg every 48 hours may be more appropriate (Kolmstetter *et al.*1997; Kolmstetter *et al.* 1998).

Treatment should continue for at least two weeks, although much longer courses may be required. Unfortunately, although metronidazole in considered effective against amoebic trophozoites and is an effective extra-intestinal amoebicide, it may be only partly effective or even ineffective against amoebic cysts

(Plumb 1996). Consequently, combination drug therapy may be needed to completely clear an infection.

Iodoquinol or diiodohydroxyquin (Yodoxine®, Glenwood Palisades, Tenafly, NJ)

Iodoquinol has been used to treat amoebiasis in humans (PDR 1997), and has been used by Dr. Barbara Bonner (The Turtle Hospital of New England, Upton, MA) and the author (CI) in several hundred specimens of approximately a dozen species of chelonians. Many reptile practitioners may have unknowingly used this drug, which is a component of Flagenase®, a metronidazole suspension from Mexico that has often found its way into the hands of United States veterinarians. Previously available in tablet form, iodoquinol may be difficult to obtain. Compounding pharmacies may been able to obtain iodoquinol powder and formulate a palatable suspension.

A dose of approximately 50 mg/kg orally once daily for 21 days has resulted in cessation of cyst shedding (not necessarily proving complete eradication) in the majority of cases. No adverse effects have been documented in humans, and necropsy of several treated animals failed to show lesions indicative of drug toxicity. Denver *et al.* (1999) reported lesions of splenitis, hepatitis and pancreatitis in juvenile black rat snakes treated with iodoquinol at 30–120 mg/kg daily for 14 days. Iodoquinol is thought to act mainly as an intestinal amoebicide, and is effective against both the trophozoite and cyst forms.

Chloroquine (Aralen®, Sanofi Winthrop, New York, NY)

Chloroquine is most commonly used to prevent and treat malaria in humans. It is also labelled for treatment of extra-intestinal amoebiasis and is effective against trophozoites (Physicion's Desk Reference 1997). It is available for either intramuscular injection or oral administration. Previous reports of the use of chloroquine in reptiles to treat *Plasmodium* infections, have quoted a dose of 125 mg/kg PO every 48 hours for three doses (Plumb 1996). In humans, a dose of 50 mg/kg per week is generally used to prevent malaria, while a dose of 50 g/kg/day may be used in treatment of malaria (Physician's Desk Reference 1997).

The author (CI) has used chloroquine in three chelonian patients at a dose of 50 mg/kg IM once weekly for three doses. One patient developed moderate ataxia in the limb used for injection after the second dose; however, normal function was regained over several weeks, and it was unclear whether the drug was the cause of the problem. Other adverse effects experienced by humans, such as vision abnormalities, were not detected, but would be difficult to establish in chelonians. In all cases, amoeba cysts were still found on faecal examination after treatment, and it is likely that chloroquine may be required in conjunction with a drug effective against cysts. Nevertheless, chloroquine is deserving of further investigation, particularly because of its convenience in chelonians as the only amoebicide that is available for IM injection.

Paromomycin (Humatin®, Parke-Davis Div., Warner-Lambert Canada Inc., Scarborough, Ontario, Canada)

Paromomycin is often used as part of combination drug therapy for treating human amoebiasis (Garcia & Bruckner 1997; Fatkenheuer *et al.* 1997). It is an aminoglycoside antibiotic that is not generally absorbed from the gastrointestinal tract. It is generally used as a lumenal amoebicide to eradicate amoebic cysts.

Paromomycin has been used in reptiles for treatment of amoebiasis and cryptosporidiosis. Pare *et al.* (1997) reported elimination of *Cryptosporidium* shedding in two gila monsters treated with 300 to 360 mg/kg paromomycin every other day for 14 days. No adverse affects were noted. In addition, Lane & Mader (1996) report that Schweinfurth used paromomycin in snakes at a dose of 25 to 100 mg daily for four weeks to eliminate amoebiasis.

The human paediatric dose of paromomycin is generally 30 mg/kg TID for 7 days (Garcia & Bruckner 1997). Paromomycin has recently been associated with acute renal failure in several feline patients in which it was used to treat trichomoniasis (Gookin *et al.* 1999). It is hypothesised that systemic absorption across a compromised intestinal mucosa may have occurred. The possibility of this toxicity should also be considered in reptiles.

Diloxanide (Fumaride®)

The use of diloxanide for treatment of amoebiasis has not been widely reported in reptiles, but the drug is often recommended in human cases. In humans, diloxanide may have fewer side effects in children than other amoebicidal drugs (Garcia & Bruckner 1997). Like paromomycin, diloxanide is a lumenal amoebicide, effective against the cyst stage. It may be used alone for treatment of asymptomatic cyst passers, or in combination with metronidazole for invasive amoebiasis (Fatkenheuer *et al.* 1997). The human paediatric dose of diloxanide is generally 20 mg/kg SID for 10 days (Garcia & Bruckner 1997).

Denver *et al.* (1999) evaluated diloxanide at 20, 40 and 60 mg/kg daily for 14 days in healthy black rat snakes and found no signs of toxicity. Its efficacy in treating amoebiasis is being evaluated at the Baltimore Zoo, in the United States at this time. Unfortunately, diloxanide is not readily available at this time in the United States.

Additional therapy

In addition to treatment with amoebicidal drugs, infected patients may also need nutritional support, antibiotics and antifungal medications. A thorough assessment of the patient must be made and amoebae treated with consideration of other possible concurrent disorders.

Elevation of ambient temperature has been shown to affect the ability of *Entamoeba invadens* to infect snakes. Jacobson *et al.* (1983) has suggested this may also be beneficial in other reptiles.

Jacobson *et al.* (1983) also advises concurrent broad-spectrum antibiotics to manage secondary opportunist Gram-negative organisms over a two-week treatment period.

Disinfection procedures are essential to prevent cage-to-cage spread of organisms or infection of animals housed after those that carry disease.

Repeated evaluation of faecal samples over a several months quarantine period is required to ensure eradication.

BALANTIDIUM AND NYCTOTHERUS

Clinical significance

Probably a normal commensal microorganism, although a variety of contradictory opinions have been published regarding

pathogenicity (Bone 1992; Mader 1996). Some animals with gastrointestinal disturbances have large numbers of ciliates in their faeces.

Diagnosis
(also see Clinical Pathology)

- Cyst forms may be misidentified as parasites.
- Cyst and ciliated (trophozoite) forms are found in faeces.
- The cyst form of *Nyctotherus*, in particular, is a large, operculated structure that may be mistaken for a trematode ovum. Finding motile adults along with cysts aids in their identification. Careful evaluation of cysts may also allow the investigator to identify cilia of the developing organism along the interior of the cyst walls.

Treatment
(also see Therapeutics)

Treatment for ciliates is often not indicated unless large numbers are present, the animal is ill and other causative agents have been ruled out. Metronidazole is effective at the same doses used for other protozoal infections.

COCCIDIANS

Clinical significance

Eimeria and *Cryptosporidium* are reported in terrestrial and semi-aquatic chelonians.

Over 30 species of coccidian are known to infect tortoises and turtles (McAlister & Upton 1989). Terrestrial chelonians infected with *Eimeria* are generally asymptomatic, although it has been suggested that infection may contribute to debility in sick animals (Barnard 1986a; Jacobson *et al.* 1994). Cryptosporidiosis is dealt with separately in Table 13.27.

Jacobson *et al.* (1994) describe intranuclear coccidiosis in two radiated tortoises (*Geochelone radiata*) associated with profound debility and muscular weakness. Haematology and biochemistry assessments demonstrated mild anaemia and renal compromise. Both tortoises underwent euthanasia and nephritis, hepatitis, enteritis and pancreatitis were evident during histopathology examination, with intranuclear developmental stages of an intranuclear coccidian parasite identified within these organs. The agent could not be identified. The same disease was reported by Garner *et al.* (1998) as affecting *G. radiata*, *Indotestudo forsteni, Manouria impressa* and *G. pardalis*.

Diagnosis
(also see Clinical Pathology)

Ovoid, sporulated oocysts of *Eimeria* are typically 10–15 µm × 25–37 µm and found in faeces. Each contains four spherical sporocysts (Barnard 1986).

Histopathology of tissues may be required to identify intranuclear coccidiosis, as faecal shedding is not always apparent.

Jacobson *et al.* (1994) were unable to demonstrate the presence of faecal oocysts in infected *G. radiata*. Transmission electron microscopy was used to identify trophozoites, merozoites, microgametes and macrogametes.

CRYPTOSPORIDIOSIS

Clinical significance

Coccidians of the genus *Cryptosporidium* have been described in turtles and tortoises, but only rarely associated with disease. Most references suggest that the organism uneventfully inhabits chelonian upper digestive tracts. Occasional anecdotal reports suggest that regurgitation and gastritis may ensue (Graczyk *et al.* 1998).

Gastritis and regurgitation were associated with cryptosporidiosis in two species of tortoise (O'Donoghue 1995). Cryptosporidiosis in *Testudo kleinmanni* was associated with parasitism of over 80% of the epithelial cells of the small intestine resulting in a diffuse chronic severe coelomitis and ascites (Graczyk *et al.* 1998). *Pseudomonas maltophila* was cultured from both the cloaca and coelomic fluid. Presenting signs included a severe oronasal discharge and the animal was thin and dehydrated. The authors propose that the *Cryptosporidia* was a contributing factor to a bacterial enteritis, septicaemia and coelomitis, which resulted in death.

Diagnosis
(also see Clinical Pathology)

Wright (1997) suggests a quarantine protocol and screening test for carriers of *Cryptosporidium* spp. using a combination of three faecal examinations three to five days apart, a 60-day quarantine (for tortoises) and a dexamethasone immunosuppression injection (2–4 mg/kg IM) followed by three repeat faecal examinations.

Oocysts (~5 µm) are most easily detected using phase-contrast microscopy after faecal flotation. They must be differentiated from yeasts and can be identified using acid-fast stains. Alternatively, immunofluorescent assays (Merifluor IFA) are more sensitive than older methods. Gastric-wash fluid or fluid collected by enema may be evaluated.

Treatment
(also see Therapeutics)

Virtually all anticoccidial agents have failed to control human and animal *Cryptosporidium* infections. However, two agents have recently shown some efficacy. Pare *et al.* (1998) reported cessation of organism shedding in gila monsters treated with paromomycin. Cranfield *et al.* (1999) report efficacy of hyperimmune bovine colostrum in treating *Cryptosporidium* in leopard geckos and monitor lizards. However, this product is not commercially available at this time.

Management in animal outbreaks is based upon isolation and removal of positive animals.

TRICHOMONAS/FLAGELLATES

Clinical significance

There are limited reports of flagellate infections associated with pathology in reptiles, except with regard to *Hexamita* in chelonians (see below). It is hard to attribute disease to flagellates, as their presence should generally be considered to be normal. However,

stressed and poorly maintained chelonians often have noticeably high numbers upon microscopy of wet mounts of faeces.

Environmental and nutritional factors, or concurrent disease and immunocompromise, may be more significant then the presence of flagellates. Herpetologists often seem to suggest that alterations to husbandry and diet encourage numbers to regress, suggesting that increased numbers are a reflection of problems in these areas.

Bone (1992) considers imbalances of normal bacterial microflora with the presence of excessive flagellates to be a significant clinical problem requiring effective and rapid treatment with antiprotozoans. Lane & Mader (1996) suggest that all flagellates appear to be commensals and non-pathogenic. However, they also suggest that excessive numbers are associated with anorexia, weight loss and unthriftiness over time.

At this author's clinic (SM), recently imported *Geochelone pardalis* and *Geochelone carbonaria* have been presented passing watery, foul-smelling and bulky faeces with large numbers of trichomonads. Often this would seem associated with selective eating of fruits as well as transportation and a recent period of neglect during shipment. In these circumstances we have seen a favourable response to antiprotozoan therapy. It would seem probable that ill health is primarily related to other influences such as starvation, an unbalanced or inappropriate diet or maintenance below the ATR of the species in question. Improvements in condition may relate more to improved fluid balance, husbandry and feeding, than to any antiprotozoan therapy given.

The individual clinician must decide the need for aggressive antimicrobial therapy. At this author's surgery, it is common for a single dose of an antiprotozoan treatment to be given in conjunction with probiotics in an attempt to normalise deranged gut flora in chelonians that are presented with apparent flagellate overgrowth.

The use of modern wormers may have some influence over the normal protozoans present as intestinal microflora. Fenbendazole has antiprotozoan activity against *Giardia* in dogs, which implies that reptiles routinely wormed with this or oxfendazole may also have any protozoan burdens reduced simultaneously.

Diagnosis

(also see Clinical Pathology)

Examination of excreta, colonic washings or other body secretions by wet-mount microscopy. Flagellates tend to be fast moving if viewed in a fresh sample. A drop of formalin or similar debilitating agent may reduce activity in order to allow flagella location and number to be ascertained, and facilitate identification.

Treatment

(also see Therapeutics)

Debilitated animals require fluid and nutritional support, with appropriate temperature, lighting and humidity. Some keepers have suggested that maintaining reptiles without diurnal temperature variations may encourage protozoan overgrowth.

High standards of hygiene, including regular disinfection of vivaria, surfaces and utensils, will restrict the spread of virulent strains.

Antiprotozoans, as used to manage *Entamoeba invadens*, may reduce overgrowth associated with abnormal and excessive blooms:

- dimetridazole (Emtryl®) 40 mg/kg orally for five days (Jacobson 1986);
- metronidazole (Flagyl®) 125–275 mg/kg as a single dose. This can be repeated in two weeks if faecal examination or clinical condition suggests that it is required (Jacobson *et al.* 1983 & 1986). Alternatively, it may be given at 20 mg/kg every other day until eradication is achieved (Kolmstetter *et al.* 1997; Kolmstetter *et al.* 1998)

HEXAMITA

Clinical significance

Hexamitiasis is described as a slowly progressing disease causing no specific symptoms. It was identified in eight species of chelonian and appeared to affect those organs that have an open connection with the digestive tract.

Clinical signs associated with advanced disease include nephromegaly, anorexia, weight loss and gradual onset of renal failure (Zwart & Truyens 1975).

Hexamita spp. must be differentiated from trichomonads (e.g. *Hexamistix* spp.). On faecal and urine analysis, both appear similar. It is unclear if some of the organisms described in earlier texts were actually commensal trichomonads.

Lane & Mader (1996) suggest *Hexamita* has pathogenic qualities only in combination with another pathogen, parasite or problem.

Diagnosis

(also see Clinical Pathology)

Renal biopsy and histopathology are necessary in order to determine whether pathology is associated with the presence of *Hexamita*-like organisms. The presence of flagellates in urine is potentially normal.

Treatment

- (also see Therapeutics) Dimetridazole (Emtryl®), 40 mg/kg orally for five days (Jacobson 1986).
- Metronidazole (Flagyl®), given as a single dose at 125–275 mg/kg. This can be repeated in two weeks if faecal examination or clinical condition suggests that it is required (Jacobson 1983 *et al.* & 1986).

METAZOAN PARASITES

All groups of reptiles are susceptible to infection by a variety of nematodes and any site may be involved. Diagnosis is dependent upon the presence of characteristic ova within faeces, or larva within faeces or tissues. Various nematode eggs and larvae can be found in chelonian faeces, and helminth parasitism is common in captive chelonians.

ASCARIDS

Clinical significance

Sulcascaris spp. are common in turtles. *Angusticaecum* spp. are common in terrestrial chelonians (Frank 1981).

Ascarids are large, relatively common parasites, often reaching 10 cm or more in length. Adults inhabit the gastrointestinal tract and shed embryonated eggs in their host's faeces. The herbivore ascarids tend to have direct life cycles. Lesions are produced either because of visceral *larva migrans* or through damage resulting from embedding of adults within the gastrointestinal mucosa.

Clinical signs are non-specific and may be a result of intestinal perforation or visceral migration and disseminated bacterial infections because of cuticle carriage of bacteria by nematode larvae during visceral migrations (Frye 1991).

Diagnosis

(also see Clinical Pathology)

Ascarid eggs can be identified in stomach washings and in faecal flotation samples. They are typically thick walled and measure 80–100 µm by 60–80 µm.

Treatment

(also see Therapeutics)

A variety of anthelminthics have been used in reptiles (Jacobson 1986) including:

- mebendazole at 20–25 mg/kg body weight, repeat in 2–3 weeks;
- thiabendazole at 50 mg/kg body weight, repeat in 2–3 weeks;
- fenbendazole at 50–100 mg/kg, repeat in 2–3 weeks;
- oxfendazole at 66 mg/kg, repeat in 2–3 weeks or as required;
- piperazine and ivermectin products are not advised.

Environmental hygiene is important in limiting reinfection.

OXYURIDS (PINWORMS)

Clinical significance

Pinworms (e.g. *Alaeuris, Mehdiella, Tachygonetria, Thaparia* spp.) have a direct life cycle and appear to be host specific (Telford 1971).

They are a common chelonian parasite:

- 75% of chelonians presented at the authors' clinic have oxyurid eggs in faecal samples (RJW/SM/JM);
- 50/71 captive tortoises in Hungary were infested (Satorhelyi & Sreter 1993);
- 4/70 United Kingdom captive chelonians presented to Holt *et al.* (1979) were infested.

Affected animals are usually asymptomatic, with parasites located within the lower digestive tract and cloaca. When numbers are moderate there is a suggestion that they may help break up faecal masses, preventing constipation (Frank 1981).

Infestation with vast numbers may be debilitating and exacerbate concurrent problems. Frank (1981) observed that heavily-parasitised animals were prone to fatal post-hibernation anorexia. However, the heavy parasite burden and death may both relate to inappropriate husbandry. Severe infestations may cause anorexia or obstruction.

Recently imported juveniles maintained in small and poorly maintained vivaria are exposed to a high level of challenge and often appear overwhelmed by what one would expect to be a non-pathogenic agent. Visceral larval migration is not described.

Diagnosis

(also see Clinical Pathology)

Oxyurid eggs have variable morphology but lack the scalloped surface and very thick walls typical of ascarids. They are often asymmetrical (D-shaped) and in some species they are oval or elongate. Occasionally, eggs are operculated at one or both ends and larvae are often present.

Adults are small but visible to the naked eye and may be present in vast numbers in the large intestine.

Treatment

(also see Therapeutics)

A variety of anthelminthics have been used in reptiles (Jacobson 1986) including:

- mebendazole at 20–25 mg/kg body weight, repeat in 2–3 weeks;
- thiabendazole at 50 mg/kg body weight, repeat in 2–3 weeks;
- fenbendazole at 50–100 mg/kg, repeat in 2–3 weeks;
- oxfendazole at 66 mg/kg, repeat in 2–3 weeks or as required;
- piperazine and ivermectin products are not advised.

Per cloacal worming of *Testudo graeca*, *Geochelone elegans* and *Testudo kleinmanni* tortoises was used to eliminate heavy burdens of adult *Oxyurid* that were not eliminated following oral dosing with fenbendazole at 100 mg/kg. Cloacal dosing with the same product, also at 100 mg/kg was suggested to have resolved the infestation resulting in clear faecal flotation screens two and four weeks later (Innis 1995).

Environmental hygiene is important in limiting reinfection.

PROATRACTIS

Clinical significance

Proatractis is an unusual parasite, with a direct life cycle, similar to oxyurids, specific to chelonians (Rideout *et al.* 1987). A single report is associated with profound disease.

Rideout *et al.* (1987) describe an outbreak of fatal disease in three *Geochelone pardalis* and eight *Geochelone carbonaria*. Non-specific clinical signs—anorexia and lethargy—preceded death. Disease was associated with a heavy intestinal burden of the viviparous pinworm *Proatractis*. Post-mortem examinations revealed pathology of the caecum and colon, and in six tortoises worms were also found in the small intestine. Eleven affected tortoises were either found dead or were presented with anorexia, lethargy and depression. Three tortoises developed mucoid diarrhoea in the terminal stages of disease.

A cause and effect relationship was not necessarily established, and the outbreak was reported to have occurred following the recent introduction of 14 wild-caught red foot tortoises (*Geochelone carbonaria*). It is not clear if a simultaneous viral infection may also have been present.

Diagnosis

(also see Clinical Pathology)

Viviparous—larvae in direct smears or Baermann apparatus preparation of faeces.

Characterised by a rosette around the buccal opening (Caballero 1971). At post-mortem large numbers of 5–10 mm worms may be found in the caecum and colon.

Treatment

(also see Therapeutics)

Optimal treatment unknown, hygiene is therefore important.

OTHER METAZOAN PARASITES

(Generally these are of unknown pathogenicity, and optimum treatment is unknown)

Flukes

Monogenetic (direct life cycle) trematodes inhabit the nasopharynx or urinary bladder of aquatic chelonians (e.g. *Chrysemys*, *Pseudemys* and *Chelodina*) and are probably non-pathogenic.

Digenetic trematodes (those for which an intermediate host is necessary) may inhabit a variety of internal organs. In terrestrial and freshwater chelonians they appear to be non-pathogenic. Frye (1991a) describes granulomata associated with flukes during visceral migrations and gives these as a reason for deep-body infections. The life cycle of flukes would appear to be indirect and further infection seems unlikely in captive terrestrial chelonians if good hygiene is observed. Fluke eggs in terrestrial species may be no more than an incidental finding.

Eggs are common in the faeces of captive chelonians in our experience, and they are not associated with specific clinical signs.

Distinctive orange or deep yellow, thin-shelled, often operculated eggs in faeces. May contain discernible miracidia.

Spirurids

Adults are present in the body cavity. Gravid females lie in subcutaneous tissue and shed larvae through the skin (Frank 1981).

Apart from skin lesions, these infestations appear to be non-pathogenic.

Dracunculid larvae may be present in skin samples (Frank 1981).

Acanthocephalans

(spiny-headed worms)

Aquatic or semi-aquatic chelonians may be heavily infested. Martin (1972) found 123/125 *Trachemys scripta* to be affected.

Frank (1981)reported no clinical signs, although Telford (1971) considered them to be potentially pathogenic.

Eggs are present in faeces, and are said to be easily identified (Frank 1981).

Larvae may be found encapsulated in the intestinal wall or other viscera.

Adults occupy the intestine.

Cestodes

(tapeworms)

Cestodes appear to be uncommon in chelonians (there appear to be no reports for sea turtles). *Ophiotaenia* occurs rarely in freshwater chelonians (Frank 1981). *Glossocercus* and *Bancroftiella* are cestode parasites of freshwater turtles (Pichelin *et al.* 1998). No pathogenic effects reported.

Thick-walled, spherical eggs of *Ophiotaenia* have visible hooklets.

NEOPLASIA OF THE DIGESTIVE TRACT

Gastrointestinal neoplasia appears to be rare in chelonians. In a review of chelonian neoplasia, Done (1996a) lists two cases of gastric carcinoma in aquatic chelonians.

Rideout *et al.* (1993) reported a series of four cases of gastrointestinal tract lymphoma in *Geochelone nigra*, and speculated that a viral or genetic aetiology may be a possibility, although no virus could be isolated from the tissues. Lesions were found most commonly in the small intestine and colon but were also seen in the oral cavity, oesophagus, and stomach.

This author (SM) has encountered plasmacytic lymphocytic enteritis and intestinal lymphoma associated with the presence of a herpesvirus in *Testudo hermanni* and *Geochelone pardalis* (McArthur 2001), and intussusception and intestinal obstruction associated with gastric and hepatic neoplasms in *Testudo graeca*.

LOWER RESPIRATORY TRACT INFECTIONS

(Fig. 11.91)

Aetiology

The information presented here is a summary only. The subject is described in greater detail in a subsequent section, dealing specifically with the aetiology of chelonian respiratory disease, its diagnosis and its management. Lower respiratory tract disease is relatively common in captive chelonians presented to this author (SM).

Causative agents include:

- viral agents;
- bacterial agents;
- mycotic agents;
- mixed and varied combinations of these.

Clinical signs

Clinical signs are not always present and are often non-specific. By the time lower respiratory tract disease is apparent it will have become well established and hard to resolve. Some of the clinical signs include:

- abnormal posture (inspiratory and/or expiratory dyspnoea may manifest as laboured breathing with the neck extended and mouth open);
- inactivity;
- anorexia;
- lethargy;
- increased or abnormal respiratory sounds;
- increased respiratory rate–especially at rest;
- concurrent disease.

History

Poor standards of hygiene, husbandry and nutrition are common.

Viral disease generally follows recent exposure to chelonians presumed to be carrying an infectious agent, however recrudescence of long-term carried disease (e.g. herpesvirus) is possible if standards of nutrition and husbandry have been poor.

Diagnosis

Lower respiratory tract may be suspected during a clinical examination but confirmation generally involves some form of imaging technique combined with histopathology, cytology or lesion culture. Any of the following investigative techniques may be helpful:

- radiography (especially craniocaudal view), CT;
- haematology and biochemistry;
- microbiology;
- cytology;
- serology;
- trans-tracheal lung wash;
- percutaneous lung wash;
- trans-carapacial lung wash following shell drilling;
- tracheal endoscopy;
- lung/lesion biopsy;
- virus isolation;
- electron microscopy;
- microbiological culture.

Treatment

Appropriate treatment depends upon the aetiology and predisposing factors. General care and husbandry should be optimised.

Therapeutic options are described in greater detail in the respiratory section later on, but include:

- isolation of affected individuals;
- supportive care (e.g. nutritional/fluids/appropriate environment);
- antibacterials;
- antimycotics;
- antivirals;
- nebulisation;
- other medications (such as atropine, diuretics, oxygen therapy and immunostimulants);
- direct application of treatment onto lung tissue (e.g. through a trans-carapacial route).

MALADAPTATION

Aetiology

Not all veterinarians consider maladaptation to be a valid diagnosis, since it may describe multifactorial disease following capture of chelonians from the wild. It may include recrudescence of carried viral disease, inappropriate nutrition, housing, stocking rates, temperature provision, etc.

Overall, the term maladaptation is used here to describe the responses of a distressed chelonian following wild capture, relocation, and introduction into an alien captive environment. The problem often progresses to secondary metabolic and other diseases.

Clinical signs

Non-specific:

- inactivity;
- anorexia;
- reclusive behaviour;
- aimless pacing.

History

- Recent wild capture.
- Recent acquisition.
- Recent change in ownership.

Diagnosis

A diagnosis of exclusion, so animals will require extensive work-up following examination and history. The diagnosis of maladaptation may arise from a lack of other more specific explanations.

Treatment

Research and provide whatever seem to be the most appropriate captive conditions for the species.

- Identify the species and consider any specific age-related requirements (juveniles and adults may have different environmental preferences).
- Address nutritional, environmental and behavioural requirements of the species.
- Consider shade placement and environmental enrichment.
- Consider whether stocking rates are appropriate. As most chelonians are solitary in the wild, consider isolating cases that are struggling.
- Consider supportive measures (e.g. oesophagostomy tube placement/force-feeding).
- Clear the animal of parasites where possible.
- Isolate animals considered potentially infectious.

METABOLIC BONE DISEASE (MBD) AND NUTRITIONAL SECONDARY HYPERPARATHYROIDISM

(Figs 11.47–11.59, 11.76–11.78)

Aetiology

Metabolic bone disease (MBD) is a condition many authors consider to be caused by dietary and husbandry mismanagement. Reports of MBD affecting most reptiles are plentiful in the veterinary literature. In this text, the term 'metabolic bone disease' is used to cover any disease or pathology of the skeletal system resulting from a failure of the chelonian body to access suitable quantities of dietary calcium, as a result of inappropriate nutritional and environmental influences.

It is plausible that genetics, activity level, congenital errors in enzyme synthesis, calcium content of egg, egg-incubation

temperature and humidity, and environmental conditions during neonatal development, may also affect bone growth and development. MBD is characterised by metabolic defects affecting the morphology and functioning of bones and it occurs in aquatic omnivorous chelonians, aquatic carnivorous chelonians, and herbivorous terrestrial tortoises (Keymer 1978a & 1978b; Jackson 1980a; Jackson & Fasal 1981; Rosskopf *et al.* 1982; Fowler 1986; Rosskopf 1986; Highfield 1990; Frye 1991a; George 1997).

In chelonians presented at our clinic, metabolic bone disease is most frequently encountered in the rapidly growing juvenile. The demands of shell growth may predispose juvenile chelonians to MBD (Donoghue & Langenberg 1996). Growth rates greater than that obtained in the wild, with associated carapacial deformities, are also common.

Adult chelonians have a vast reserve of calcium within the shell of the carapace and plastron. It would appear that hyperparathyroidism is used to maintain blood calcium levels where light exposure, vitamin D activation, dietary Ca:P ratios and dietary calcium are inadequate. Undesirable effects of chronic hyperparathyroidism are discussed elsewhere.

Secondary nephropathy appears to be related to chronic hyperparathyroidism. The reader is also referred to the physiology, nutrition, husbandry and nutritional disease sections of this book, where more extensive detail is given.

Aetiological factors:

- dietary deficiency of calcium;
- dietary deficiency of suitable vitamin D;
- feeding a diet with an unsuitable Ca:P ratio;
- lack of exposure to appropriate ultraviolet light;
- disruption of vitamin D metabolism due to renal, hepatic, intestinal or parathyroid disease, possibly dietary excess of protein during growth periods;
- anything predisposing to anorexia (e.g. inappropriate environment provision).

Dietary deficiency of calcium

Specific calcium and vitamin D requirements are poorly determined for most reptiles. Dietary requirements of calcium vary with species, and are increased during periods of growth and female reproduction. Highfield (1988) suggests a high requirement during the first eight weeks of chelonian life when many species double their weight.

Donoghue & Langenberg (1996) suggest that most reptiles require a dietary calcium level between 1.8–3 mg/kcal (0.6%–1% DM), phosphorous levels of 0.5%–0.8% and a calcium phosphorous ratio (Ca:P) between 1:1–2:1. Their vitamin D recommendation was 200–1000 IU/kg DM. (Maximum tolerances are given as 2.5% DM for calcium, 1.6% DM for phosphorous, and 5000 IU/Kg DM vitamin D.) They also report early results of trials involving the growth of *Geochelone* spp. which they say have 'done well' on diets containing 1.4% calcium and 0.7% phosphorous over a three-year period.

Some foods, such as beet greens, kale and spinach contain good levels of calcium but unfortunately a proportion is bound as insoluble calcium oxalate, so that it is physiologically unavailable. In moderate amounts these foods are well tolerated, however, and many authors, such as Burgmann *et al.* (1993) and Donoghue (1995c) explain that they are still good sources of calcium despite their oxalate content.

Rhubarb leaves contain oxalate to excess, and should not be fed to reptiles (Frye 1991b).

Dietary deficiency of suitable vitamin D

The dietary need of vitamin D in chelonians does not seem to be well understood. It would appear that dietary vitamin D levels themselves do not dictate plasma vitamin D levels in many reptiles, and other factors such as UVB irradiation are also important. According to Highfield (1988), chelonians in their natural habitat are unlikely to suffer hypovitaminosis D_3, unless deprived of access to sunlight.

The role of light in the activation of vitamin D is briefly described in the Physiology section, where the hormonal control of body calcium levels is summarised.

Authors such as Ullrey & Bernard (1999) give evidence to suggest that access to high levels of oral vitamin D may be of less importance in many reptiles than provision of suitable UVB. Suitable UVB is said to increase activation of vitamin D precursors or facilitate gut absorption within the animal itself. It is possible that UVB increases body levels of vitamin D binding proteins through stimulation of the pituitary pineal axis, and so increases vitamin D levels through increasing the percentage of dietary vitamin D precursors absorbed.

Feeding a diet with an unsuitable Ca:P ratio

Fowler gives a simple model suggesting that calcium and phosphorous ions are soluble, and in equilibrium with various forms of insoluble calcium phosphate in the diet. An excess of either ionised calcium or phosphate will drive the equation below to the right. It is not clear how ionised or available calcium will alter with variations in dietary protein levels, because a degree of protein binding must be assumed to occur:

$$3Ca^{2+} + 2PO_4^{3-} \leftrightarrow Ca_3(PO4)_2$$
$$(\text{Soluble}) \leftrightarrow (\text{Insoluble})$$

The diet of wild herbivorous tortoises from desert habitats has been examined by a variety of authors, including Highfield (1998), and is proposed to have a calcium phosphorous ratio of 3.5:1–8:1. The wild diet of *Testudo hermanni* and *Testudo graeca* has a Ca:P ratio of 3.5:1 (Highfield 1996). Zwart (1987) suggests that a dietary ratio of 1.2:1 is commonplace amongst chelonians with metabolic bone disease. The ideal ratio of calcium to phosphorous in the diet can therefore be concluded to be greater than this, presumably in the range of 3:1–6:1.

Dietary errors with chelonians often involve too much dietary phosphorous. This causes calcium in the diet to become bound as insoluble calcium phosphate. Decreased calcium availability results in increased parathyroid activity to compensate (Donoghue & Langenberg 1996). Some green vegetation has a high level of phosphate and chelonians fed on this can benefit from the addition of a calcium rich supplement such as Nutrobal® (Vetark Animal Health), calcium lactate, grated cuttlefish or ground eggshell.

Highfield (1996) gives adverse Ca:P ratios of some foods he considers unsuitable for juvenile chelonians as: peas at 1:3; broad beans at 1:6; mung sprouts at 1:3. These are in general agreement with the figures of Donoghue & Langenberg (1996) and Donoghue (1996) given earlier, in the dietary section.

Highfield (1996) suggests that some dog foods may have a Ca:P ratio as high as 1:44.

Lack of exposure to appropriate ultraviolet light

Cholecalciferol (vitamin D) in mammals is derived from cholesterol because of exposure of skin to suitable ultraviolet radiation. The UVB requirements of specific reptiles, such as chelonians, are not yet fully understood, but many authors consider UVB photoexposure to be central in the prevention of MBD (Boyer 1992c; Harcourt-Brown 1996; George 1997). The suitability and role of artificial lights in captive reptile husbandry have been examined by Allen (1988), Gehrmann (1987) & (1994), Allen *et al.* (1994) and Brice (1995). Ideally, sunshine is the best and most suitable source of UVB for vitamin D activation. In temperate latitudes, such as the United Kingdom, supplementation with artificial sources of UVB is appropriate and advised.

Divers (1996b & c) discusses the suitability of various brands of vivarium lighting available in the United Kingdom with respect to UVB. He favours the use of lights emitting the spectrum, assumed to be 290–320 nm, which activates vitamin D_3 (Divers 1996c). Effective lights suitable for most chelonians include Powersun®, Zoomed; Reptisun 5.0®, Zoomed; Iguana Light UVB®, Zoomed; Tru-lite®, Durolite Corporation; Ultravitalux®, Osram; Active UVB®, Rainbow Rock. Most brands also include light in the visible spectrum (Divers 1996c & 1999).

Donoghue & Langenberg (1996) explain that even relatively photophobic turtles that naturally shelter from sunlight may still benefit from careful UVB exposure. It would seem appropriate that all United Kingdom captive chelonians should be provided with suitable UVB lighting, as good sunny weather cannot be guaranteed. If a chelonian species is considered to be nocturnal or photophobic, care should be taken to avoid unnecessary stress to the animal through excessive, forced UVB exposure. Whenever light supplementation of any type is provided, I advise the use of annual day-length tables and timing switches as discussed in the section on Reproductive Disease.

Boyer (1992c) points out that glass and perspex will filter the UVB output of reptile lights. Nothing should come between the reptile and the light source. Lights should be replaced frequently. Boyer recommends every two years and Divers (1996c) points out that useful UVB emissions may be absent after as few as six to nine months. Six months is normally the limit given by manufacturers.

Blacklights such as (Blacklight®, Sylvania) emit little visible spectrum, but good levels of 290–320 nm. However, there is some evidence to suggest that the use of unsuitable Blacklights has resulted in a pruritic conjunctivitis and cataract formation (Fletcher 1994; Scaife 1994) and these lights may require staff and keeper eye and skin protection to be made available. Divers (1996c) suggests that this may be due to high levels of UVC emission.

In the United Kingdom, both complications and benefits of various lighting arrangements have been described (Fletcher 1994; Scaife 1994; Harcourt-Brown 1996; Divers 1996a).

Excess dietary protein

The interactions between dietary protein and calcium metabolism in chelonians remain poorly understood. Margen *et al.* (1974) link increased dietary protein in man with increased renal excretion of calcium, but it is not clear if a similar relationship exists in chelonians. Accelerated and carapacial deformities associated with excessive accelerated growth are described by a variety of authors including Jackson (1976), Lambert (1986 & 1988), Rosskopf *et al.* (1982) and Divers (1996b).

Carapacial deformity manifesting as pyramiding of scutes appears to be the result of accelerated growth during the very early stages of life. In pyramiding, the central scute grows at a differential rate to the outer scutes, and therefore it is the very young animal that appears to have grown unsuitably. Whilst it has not yet been conclusively studied there are various hypotheses as to why this may be so:

- If accelerated growth during early life is combined with inadequate available dietary calcium, hyperparathyroidism and metabolic bone disease appear to occur.

- When combined with adequate UVB provision and a suitable vitamin and mineral supplement, the condition of pyramiding appears to be free of the consequences of hyperparathyroidism. This suggests that pyramiding is not so much related to calcium metabolism as juvenile growth rate.

- Pyramiding may be the result of inadequate humidity provision during early growth. The effect of humidity during early growth is currently being studied at the University of Vienna. It has been proposed that suitable humidity provision during early growth results in smooth carapaces. The idea behind this is that young tortoises in the wild normally hide in the vicinity of bushes, and sleep in earth crevices, where humidity is high (Meyer 2002: personal communication).

Prevention of accelerated growth and pyramiding of the scutes may involve a combination of reducing food availability (e.g. alternate-day feeding/reduced feeding times), controlled photoperiod, humidity and temperature provision, and enforcement of a short hibernation period, even in yearling hatchlings. Dietary levels of calcium, phosphorus and vitamin D should all be carefully controlled. All these strategies together encourage a slow and controlled rate of growth.

Less common causes

Diseases of the kidney and liver may interfere with calcium absorption if vitamin D activation is impaired:

- Renal disease would be expected to interfere with phosphate excretion and calcium resorption.

- Diseases of the small intestine may decrease absorption of calcium. The absorption of calcium is also reduced in the absence of bile salts, which facilitate its uptake (Fowler 1986).

- The solubility and hence availability of calcium in ingesta is influenced by the acidity of gastric contents and the presence of calcium-binding substances such as oxalate, present in plants such as spinach.

Inappropriate temperature provision

In combination with other factors, Burgmann *et al.* (1993)

propose that proper function of the digestive tract of the green iguana is impaired when the temperature is below 21°C. This is because enzymatic activity and microbial degradation of food are impaired and nutrients therefore fail to be absorbed. A parallel situation occurs in chelonians, which are also hindgut fermenters (Baillien & Schoffeniels 1961; Fox 1961; Gilles-Baillien 1970; Guard 1970 & 1980). Dawson (1975) suggests that reptiles, including chelonians, require a temperature at or near their preferred body temperature for efficient digestive function.

Clinical signs

Various presentations are possible, depending upon the age and species of animal. The presenting signs of metabolic bone disease appear to differ between semi-aquatic turtles and tortoises, juveniles and adults (Jackson 1991).

In the semi-aquatic turtle, the main finding is 'soft-shell' (Boyer 1996b). The shell is weakened to the extent that it is relatively easily compressed by finger pressure (Jackson 1991). According to Boyer (1996b), hatchling chelonians fed an adequate diet usually develop a firm shell within the first year. In growing chelonians, a distorted shell with pyramid growth of the scutes is the usual presentation of metabolic bone disease. Such tortoises may have a characteristic hollow sound when tapped. Occasionally this is accompanied by excessive and distorted growth of the rhamphotheca (Boyer 1996b).

Scott (1992) comments that many of the different presentations of soft and lumpy shells are just different degrees of the same problem.

Adult chelonians have large reserves of calcium in the bones of the carapace, plastron, and limbs and so resist hypocalcaemia for longer periods through increased parathyroid activity. Consequently, it would appear that adult terrestrial tortoises are more likely to succumb to non-specific metabolic diseases associated with chronic nutritional secondary hyperparathyroidism, than to a hypocalcaemia, abnormal shell growth and soft-shell. Hyperparathyroidism may be associated with diseases such as metastatic calcification, renal failure and deranged lipid metabolism.

Burgmann *et al.* (1993) suggest that hypocalcaemia may affect a spectrum of physiological processes. Captive chelonians suffering deranged calcium metabolism may present with signs including dystocia, anorexia, cloacal organ prolapse and abnormal digestion or constipation, all of which appear to have a strong association with MBD.

Finlayson & Woods (1977) describe a rupture of the aortopulmonary trunk of a Spanish semi-aquatic turtle (*Mauremys caspica leprosa*) associated with thickened aneurysmal arteries. Both lipid deposition and metastatic calcification were present. This was potentially a result of hyperparathyroidism.

Osteomalacia

Osteomalacia is the adult failure of bone calcification (Boyer 1996c). It is a condition in which primarily cancellous bone is softened, with decreased density. Supporting bones soften and weaken. Mineralisation fails to keep up with mineral resorption. Radiography demonstrates a loss of bone density, thinning of cortices, coarsened trabecular pattern, mottled radiolucent areas, folding fractures and bowed bones (Fowler 1986).

Osteoporosis

In osteoporosis, resorption of osteoid exceeds formation. This results in a decrease in the organic matrix of bone and therefore the density of bone. Bones are light, brittle and fragile and there is a loss of bone mass. In young animals, ossification of cartilage is delayed (Fowler 1986). Radiography demonstrates thinning of the cortices and an increased medullary cavity. Trabeculae become more pronounced as they become fewer.

Rickets

Rickets is the failure of mineralisation of osteoid or cartilaginous bone matrix, similar to osteomalacia. It is often associated with bowing of long bones and widening of metaphyses (Fowler 1986).

Fibrous osteodystrophy

Osteoclastic resorption of osteoid is replaced by highly cellular connective tissue. In higher vertebrates, fibrous osteodystrophy is often associated with mandibular pathology. In juvenile chelonians carapacial deformities such as pyramiding of the scutes and over-development of the rhamphotheca (upper jaw) are more common.

Hypocalcaemia

The clinical significance of low total blood calcium levels is hard to assess unless calculations or determinations are able to identify bound and ionised calcium. In the United Kingdom, most reported chelonian calcium values are total calcium, which does not inform the clinician how much is physiologically available. Ionised calcium values are available from some laboratories or can be measured using commercial electrolyte machines such as the I-stat® (Heska) or the Easylite calcium (Na/K/Ca/pH)® Medica Corp, Bedford MA).

Hypocalcaemia followed parathyroidectomy in *Testudo graeca* (Oguro *et al.* 1974). Neither tremors nor tetanic convulsions were observed in the parathyroidectomised animals, although serum calcium concentrations fell to about 60% of that of the controls over a three-week period. Significant alterations in serum inorganic phosphate were not observed following parathyroidectomy. This may be due to the effect of bladder equilibration of excretion fluids, described in the Renal Disease section of this book. The possibility exists, though, that removal of parathyroid-secreting tissue may have been incomplete, as cells may be distributed elsewhere, such as in the lung (Clarke 1965).

Reptiles such as the green iguana (*Iguana iguana*) often demonstrate flaccid tetany because of a hypocalcaemia associated with MBD (Boivin & Stauber 1990; Bone 1992; Lawton 1992; Boyer 1996c; Campbell 1996a; Done 1996b). It is however probable that the chelonian shell acts as a large reservoir of calcium in adults and, unlike lizards, allows them to avoid hypocalcaemia by mobilisation of this calcium through parathyroid activity. I (SM) have seen no reports of generalised flaccid tetany associated with hypocalcaemia in chelonians, but I am suspicious that weak and debilitated cases may relate to hypocalcaemia and/or potassium abnormalities. Historically, little investigation into blood ionised calcium levels has been done, and this is an area deserving further study in the next few years.

Ionised calcium values in *Testudo* spp. presented at this author's surgery (SM) are generally in the range of 1–2 mmol/l. Animals

with signs consistent with hypocalcaemia associated with nutritional secondary hyperparathyroidism appear to have ionised calcium values less than one 1 mmol/l. This author regards animals with ionised calcium levels less than one 1 mmol/l as in need of urgent calcium rescue. As the body has extensive homeostatic mechanisms geared up to maintain ionised calcium within normal levels, animals with significantly low values are decompensated and have exhausted their compensatory mechanisms. In-house laboratory equipment suitable for the measurement of ionised calcium is illustrated.

Cloacal organ prolapse, dystocia, and other conditions where muscular weakness precipitates disease are commonly associated with inappropriate diet and MBD at our clinic, and have been associated with MBD by other veterinarians (Boyer 1992b). This link is best assessed using ionised calcium values.

Campbell (1996a) suggests that total serum calcium values, using North American units, of 2–5 mg/l are possibly normal in some chelonian species. In the United Kingdom, values of 4–6.5 mmol/l would be typical March–July figures for our normal female patients. We have not seen a male tortoise with a plasma calcium level exceeding 3.1 mmol/l, or an immature female exceeding 3.6 mmol/l.

Hypophosphataemia

Hypophosphataemia often appears to accompany a normocalcaemia in chronic, debilitated chelonians maintained with inadequate light and nutrition and without calcium supplementation. It would seem likely that this hypophosphataemia relates to a period of starvation or hyperparathyroidism. Hyperparathyroidism would result in shell reabsorption, decreased phosphate absorption from the gut and increased phosphate excretion at kidney level.

Radiographic evidence of osteomalacia, osteoporosis, rickets and fibrous osteodystrophy translate into a variety of clinical presentations.

Juvenile animals

- Soft shell.
- Parrot beak.
- Flattening of the pelvis resulting in splayed hind limbs and abnormal gait.
- Pyramiding of scutes (although this can be a sign of inadequate humidity provision and can occur without MBD).
- Alterations in carapace conformation of juveniles. The inadequately calcified carapace is deformed by muscular forces associated with the pelvic and pectoral muscle masses. The caudal carapace is often pulled into a grossly and typically abnormal shape if the animal is experiencing MBD during the first few years of life, when growth is rapid.

Adult animals

- Muscular weakness.
- Cloacal organ prolapse.
- Egg retention in gravid females.
- Long-bone fractures, as seen in lizards with MBD, are not common.
- Collapse of the humeral head in *Testudo* spp. has been seen several times by this author (SM) but septic arthritis must be considered an important differential diagnosis in such cases.
- Hyperparathyroidism may result in renal compromise and degenerative hepatic lipidosis.

Semi-aquatic animals

Semi-aquatic animals often demonstrate extensive secondary erosive infections of the plastron and carapace. These often appear to relate to poor water quality combined with poor shell structure. Such animals are often soft, weak and malodorous (Fig. 11.67).

History

- Unsuitable dietary provision.
- Lack of exposure to adequate UVB, natural or artificial.
- Possible filtration of useful UVB by plastic or glass screening.

Diagnosis

- History.
- Clinical appearance: typical shell, head, and limb abnormalities may be present.
- Blood biochemistry: hypocalcaemia, especially with respect to ionised calcium.
- Radiography: decreased definition of bony structures such as the pelvis, proximal and distal limbs.

Clinical signs

As discussed earlier.

Radiography

Jackson & Fasal (1981), Fowler (1986), Jackson & Sainsbury (1992) and Silverman & Jansen (1996) describe radiographic changes apparent in chelonians with metabolic bone disease. Radiographic manifestations of MBD include severely reduced bone opacity, occasional soft tissue swelling in the neck and forelimbs and decreased radio density of bony tissues such as in the pelvis and limbs, sometimes to the extent that the animal is difficult to radiograph. Pathological fractures may be visible.

Jackson & Fasal (1981) recommend close examination of the pillars (cranial and caudal edge of the bridge connecting the plastron with the carapace) or axillary/inguinal scutes for evidence of radiolucency.

Diet evaluation

The diet of captive chelonians should be closely scrutinised during examination and assessment. It is important to realise that a tortoise may not actually be eating the foods a client is offering and this too should be investigated in order to prevent misinterpretation of information.

A suitable diet should have:

- suitable Ca:P ratio of 1.5–2:1;
- suitable amounts of both calcium and phosphorus (Ca: 0.6%–1% DM, P: 0.5–0.8% DM);
- appropriate mineral and vitamin supplementation (e.g. Nutrobal®, Vetark, UK) daily.

A suitable diet should *not* have:

- calcium-binding foods (e.g. beet greens, kale, spinach);
- meat in a herbivore diet.

A dietary Ca:P ratio of 1.2–1.5:1 is suggested by Jackson (1991) and 1.5–2:1 by Scott (1992).

UVB evaluation

It is hard to give specific advice, but it is important to assess the type of artificial light provided, if any. Some keepers do not provide any source of ultraviolet light. Different brands of light emit very different spectra and intensities of visible light: UVA: 320–400 nm and UVB: 285–320 nm (Gehrmann 1994). Light intensity, photoperiod and quality are all-important and require individual assessment. There is significant evidence to suggest that dermal vitamin D_3 activation occurs through exposure to UVB in both humans and reptiles (Allen *et al.* 1994).

Factors to be considered include:

- type and brand of any light source;
- positioning of the light;
- replacement period;
- competition for basking areas;
- photophobia induced by intense lighting.

This author suggests the use of Reptisun® fluorescent strip lights (Zoo-Med, USA), and suggests annual replenishment, positioning within six inches of the animal and a photoperiod based upon latitude and annual variation, as given in table form in the section dealing with reproductive function. This is similar to the advice given by Divers (1996b & c) and Divers (1999). The emission spectrum of lights commonly available in the United Kingdom is examined by Divers (1996b) and those in the United States by Gehrmann (1987), Allen *et al.* (1994), Gehrmann (1994) and Divers (1996b). Certain plastics allowing passage of UVB from natural sunlight are now commercially available (Solacryl SUVT®, Polycast, Stamford, CT) and these should be strongly considered in the design of any reptile enclosure (Allen *et al.* 1994).

Clinical Pathology

Blood calcium and inorganic phosphate levels

Blood calcium and inorganic phosphate levels may help, but little data is currently published on blood values in uncomplicated chelonian MBD cases. In addition, the lack of availability of ionised calcium levels makes these figures potentially unrepresentative of metabolically available calcium. A further complication is the supplementary role of the bladder and kidneys in maintaining blood calcium and inorganic phosphate levels.

Low calcium values are strongly suggestive of metabolic bone disease, especially if accompanied by a normal or elevated albumin level.

In good health, the Ca:P ratio of many vertebrates is around 2:1 (Fowler 1986).

Elevated values may occur during vitellogenesis in females. The lack of ability to test for ionised calcium is an important handicap and means even females with apparent hypercalcaemia through raised total calcium may still be deficient in levels of available ionised calcium. Interpretation of blood levels must therefore take into account several factors, including standards of husbandry and nutrition. Hyperparathyroidism is therefore a subjective diagnosis if there is no access to suggestive radiographic evidence, serum PTH levels and ionised calcium levels.

Wronski *et al.* (1992) used shell biopsy as a method of comparing different populations of desert tortoises (*Gopherus agassizii*). They used a combination of shell thickness, shell porosity, osteoid surface and osteoid seam width as their parameters. However, they concluded that dermal bone on the periphery of the carapace was a poor sampling site for evaluating the effects of dietary or environmental conditions in calcified tissues in desert tortoises.

Treatment

Correct dietary and environmental causes

- Correct inappropriate Ca:P ratio of the diet, removing unsuitable items and balancing the diet.
- Restore vitamin D control of calcium metabolism by improving UVB light provision.
- Restore blood calcium levels with parenteral calcium if clinically hypocalcaemic.
- Supplement oral vitamin D_3 (calcitriol, Rocaltrol®, Roche during initial stabilisation and Nutrobal®; Vetark, UK, during medium- to long-term recovery).
- Beware of excessive vitamin D supplementation and metastatic mineralisation. Adjust/reduce supplements as the case stabilises.
- Calcitonin injections may prove helpful in terminating hyperparathyroidism, but further research into this is required.

Correct dietary problems

According to Jackson (1991), most semi-aquatic turtles demonstrating soft-shell have usually been offered a diet of fish fillets or mincemeat without additional supplementation with minerals and vitamins. This diet has an adverse Ca:P ratio. Dietary correction requires a more balanced approach to feeding, avoiding red meats and fish fillets. Nutrobal® (Vetark, UK) has a Ca:P ratio of 46:1 and can be added to meat-based foods when given to omnivorous species. A small amount of dog food once a week in omnivorous species will provide additional vitamin D_3. Full-spectrum lighting is always advised, unless for some reason the animal becomes distressed or photophobic.

Burgmann *et al.* (1993) suggest that a high-available-calcium diet for a herbivorous reptile, having a positive Ca:P ratio, can be achieved by including Swiss chard, kale, beet greens, escarole, parsley, watercress, green beans, broccoli and flowers such as roses and nasturtiums, carnations and hibiscus. Care should be taken to vary the diet and prevent addiction to foods such as cucumber and iceberg lettuce, which contain limited nutrients. Wild foods and green-leafed foods in combination, offer excellent nutrition.

If an ideal diet and appropriate irradiation are provided then supplementation with a calcium balancer and vitamin D should not be necessary. In practice this is often hard to achieve. Occasional dosing with calcium and vitamin D (Nutrobal®, Vetark, UK) is recommended by this author, especially for growing tortoises, and always in combination with full-spectrum lighting. Oyster shells and cuttlefish are excellent sources of calcium carbonate that can be used to improve adverse Ca:P ratios.

Correct environmental problems

Sunshine is superior to artificial light for patient well-being and vitamin D activation. On sunny days the tortoise should be maintained outside; on dull days UVB vivarium lighting should be provided. Emissions should be strong in the 290–300 nm range

(Burgmann 1993). We currently advise the use of fluorescent tubes (Reptisun®, Zoomed) in captive chelonians presented at our surgery, unless they are photophobic, or omnivores where vitamin D is available in an appropriate diet. A day length pattern similar to the location of origin should be provided.

Temperatures should be maintained within the preferred range for the species to facilitate metabolism, digestion and well-being.

Restriction of excessive growth can be achieved through controlled hibernation and aestivation, and a season-based vivarium environment with encouragement to exercise.

Vitamin D_3 orally

A suitable dose of oral vitamin D_3 for the tortoise has not yet been determined. There are anecdotal reports of excessive soft-tissue mineralisation when it is provided to excess in herbivorous reptiles (Frye 1991b; Burgmann 1993), however this is unlikely to occur if aggressive supplementation is restricted to the initial stabilisation period.

At our clinic we provide oral vitamin D_3 as dihydrotachysterol (AT.10® Sanofi/Winthrop) or calcitriol (Rocaltrol, Roche) at an empirical dose of one drop per kg (or part thereof) daily for one to three days during initial stabilisation. We then give one drop per kg (or part thereof) on alternate days, depending on the severity of the condition. Treatment with this form of vitamin D_3 rarely exceeds two weeks. Simultaneous provision of an oral calcium/vitamin D balancer (Nutrobal®, Vetark) is continued for at least the season, in combination with long term improvements of light provision, nutrition and general husbandry.

Parenteral calcium

As chelonians rarely present with paralysis associated with MBD parenteral injection may not be justified unless a true hypocalcaemia relating to ionised plasma calcium is present. Parenteral intracoelomic calcium injections, at a dose rate of 100 mg/kg 10% calcium gluconate, have been advised in other reptiles with hypocalcaemia and tetanic spasm associated with metabolic bone disease (Bennett 1996).

The main indication in diseased chelonians for parenteral calcium administration is dystocia in an animal that may be hypocalcaemic. If it seems likely that the patient is hypocalcaemic then calcium gluconate, calcium borogluconate or even calcium lactate (10% solution) can be given at 0.5–1.0 ml/kg body weight (to complement any oxytocin given later). Zwart (1992) advises injection of calcium borogluconate at 10 mg/100 g.

Salmon calcitonin

Belanger *et al.* (1973) used 1 ng/g synthetic salmon calcitonin to block the osteolytic effect of parathyroid extract in *Pseudemys scripta*. An empirical dose of salmon calcitonin (Miacalcin®, Schering Plough) at 50 IU/kg IM once a week, for two weeks, is suggested by authors such as Mader (1993) and Bennett (1996) for the green iguana. Mader (1993) stresses the importance of achieving normocalcaemia before commencing treatment and suggests simultaneous vitamin D_3 and calcium therapy. He further suggests that recovery time in iguanas so treated for MBD is reduced from four to six months to four to six weeks.

This author (SM) currently treats nutritional secondary hyperparathyroidism in *Testudo* spp. using a standard calcium rescue protocol described earlier with respect to vitamin D supplementation in combination with calcitonin injections. Practical in-house ionised calcium assessment has meant that such animals can be carefully monitored throughout the stabilisation process. Calcitonin is injected at a dose of 50 IU/kg after at least three days of oral vitamin D/Ca supplementation and ionised calcium levels are then monitored for the following three days. Calcitonin is regarded by many physiologists as the negative feedback messenger terminating parathyroid hormone manufacture and therefore a single dose may dramatically improve the outcome of cases of chronic nutritional secondary hyperparathyroidism.

METASTATIC CALCINOSIS/ PSEUDOGOUT

Aetiology

Fowler (1986) and Frye (1991b) suggest that feeding excessive amounts of monkey, dog or cat foods, with associated high vitamin D content, can result in heavy and perhaps even fatal soft-tissue mineralisation of tissues such as the kidneys, heart, joints, arteries and central nervous system. Care should be taken when offering these products to reptiles and their suitability for most captive chelonians is questionable.

According to Burgmann *et al.* (1993), chronic excess of vitamin D can even result in bone resorption and weakness in combination with hypercalcaemia in the green iguana. However, there is some evidence that metastatic calcification is not pathognomonic for hypervitaminosis D, as it has been seen to occur in situations where plasma and dietary vitamin D levels were unremarkable (Ullrey & Bernard 1999).

Richmann *et al.* (1995) describe iguanas with concurrent metastatic calcification and metabolic bone disease (MBD), displaying pathological fractures, hypovitaminaemia D and other signs of MBD. Metastatic calcification should not necessarily be taken as proof of hypervitaminosis D, and other factors such as parathyroid hormone and calcitonin levels may also be important.

Diagnosis

Metastatic calcinosis is usually a post-mortem finding, although radiographic and ultrasonography findings may be suggestive. Measurement of blood parathyroid hormone and calcitrol levels may prove helpful in prevention in the future.

Barten (1982) describes fatal metastatic mineralisation associated with chronic vitamin D toxicity in a red-footed tortoise (*Geochelone carbonaria*). This animal presented with anorexia of two weeks' duration, lethargy and absence of urine and faeces for two months. An inflamed oropharynx was observed and the owner had reported occasional vomiting.

The tortoise died soon after presentation and post-mortem examination revealed inflammation and mineralisation to be widespread throughout the coelomic cavity. Histopathology demonstrated metastatic mineralisation in most soft tissues containing smooth muscles including oviducts, ovarian stroma, stomach, small intestine, bladder, muscular arteries of the heart, lungs and kidneys. A suppurative hepatitis, mild interstitial pneumonia and moderate non-suppurative enteritis were present. The

source of vitamin D in this case was commercial cat food that had been fed over several years. Barten comments that it took seven years for clinical signs to appear.

Finlayson & Woods (1977) describe the pathological features of arterial lesions in 200 exotic reptiles. Calcification of ventral aortae and pulmonic arteries was observed in a female star tortoise (*Geochelone elegans*) and a rough turtle (*Geoemyda punctularia*). Vascular calcification in reptiles has been attributed to hypervitaminosis D (Wallach 1966 & 1971).

Finlayson & Woods (1977) also describe the rupture of the aortopulmonary trunk of a Spanish turtle (*Mauremys caspica leprosa*) associated with thickened aneurysmal arteries. Both lipid deposits and metastatic calcification were present. The authors suggest that vascular calcification may also be a consequence of hyperparathyroidism and that nutritional myopathy and metastatic calcification may occur concomitantly in animals suffering a relative or absolute calcium deficiency.

Frye & Dutra (1976) describe articular pseudogout in a two-year-old red-eared slider (*Trachemys scripta elegans*). The turtle presented with firm, macular swellings of the legs involving the radiohumeral, radiocarpal, femurotibial and tibiotarsal joints. It experienced difficulties in locomotion. On palpation both plastron and carapace were easily deformed. Most joints contained radio-opacities, and the turtle was euthanased with a tentative diagnosis of gout. The joint tophi were the only gross lesions seen at necropsy, and these were found to be hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. The aetiology is not clear, but presumably involves the effects of hyperparathyroidism and diet resulting in joint precipitation of the salt described.

Treatment

The outcome of the cases described above was generally fatal and efforts are best made at prevention. As research in calcium and vitamin D metabolism improves our understanding of normal care requirements, the information should be made available to keepers.

Dehydration and its influence upon solubility index should be considered in the management of all debilitated chelonians and efforts should be made to reduce the possibility of iatrogenic metastatic mineralisation during the veterinary management of debilitated cases.

POSTERIOR PARESIS OR WEAKNESS

Aetiology

Whilst posterior paresis is a relatively common presentation, there seems to be no single aetiological cause. The more common of these are:

- herpesvirus;
- bacterial and fungal infections;
- visceral and spinal gout;
- neuropathies, such as chronic pesticide toxicity from inadequately washed food;
- endocrinopathies, such as follicular stasis (hyperoestrogenism) and hyperparathyroidism;
- hypocalcaemia through osteoporosis of the spine and/or vertebrae;
- heat damage;
- frost damage;
- gravidity;
- renal disease;
- cystic calculi;
- obstipation and impaction;
- obesity;
- toxicosis from the putrefaction products of ingesta when animals with loaded digestive tracts are chilled.

Clinical signs

- Posterior paresis/hind limb weakness.

History

Depends upon aetiology

Diagnosis

A complete work-up is always indicated, to include:

- history;
- physical examination;
- blood biochemistry and haematology;
- diagnostic imaging techniques;
- specific additional investigations, such as histopathology;
- viral investigations (PCR/serology/isolation/EM).

Treatment

Treatment will depend upon aetiology:

- remove any underlying cause;
- treat any presumed or known infection;
- improve all aspects of nutrition and husbandry;
- provide a period of long-term convalescence;
- consider euthanasia if poor response to long-term treatment.

POST-HIBERNATION ANOREXIA (PHA)

The phrase 'post-hibernation anorexia' is misleading. Anorexia is a sign associated with a large number of chelonian husbandry problems and almost any acute or chronic disease. The reader is referred to the section describing normal care of captive chelonians, viral disease (especially stomatitis), follicular stasis, renal failure, gout, anorexia, rat bite trauma, sight and frost damage. Animals presented with, or generally described as having, 'post-hibernation anorexia' benefit from a complete assessment of their background and previous care, and a full clinical evaluation. This must be followed by management of all the conditions from which they may be suffering, in parallel with management for chronic dehydration.

Sadly, many United Kingdom captive chelonians are still inadequately maintained, and are not carefully managed during their winter hibernation. Many keepers wrongly feel reassured when their chelonian is apparently not adversely affected by repeated years of being placed in a back garden all summer, and then housed in a box all winter. They wrongly assume that nothing more in terms of environment (e.g. heat, light and photoperiod UVB exposure) is necessary.

Animals presented with post-hibernation anorexia have often been seriously deprived of heat, light, water and food. Most will be seriously dehydrated, whether they reveal specific clinical signs of this or not. In combination, such neglect will inevitably compromise the health of any captive chelonian.

Few animals of any class or order can be placed in a box without water for periods of six months and expected to survive. The concept of six-month water deprivation in a tortoise often amazes keepers, to whom it has not occurred that lack of water from October through to April will cause dehydration.

Lawrence (1987b) stated that animals that had not eaten within 7 weeks of rising, with or without treatment, should be given a very poor prognosis. Lawrence also suggested that tortoises that fail to drink within ten days of awakening from hibernation require veterinary intervention with fluids. This author (SM) would support Lawrence's advice, but would also actively seek data on bathing and urination in the post-hibernation period. This author generally uses urination (fluid output) as a prognostic indicator in preference to fluid input data from the client.

Most animals presented at the author's clinic will have been anuric (not producing any urine at all) for anything up to six weeks. Once the animal has urinated more than once, it is a good candidate for stabilisation using the protocol detailed below. Failure to urinate more than once within six weeks of hibernation carries a very grave prognosis.

The role of the lower urinary tract in water recycling and conservation has already been covered and the point at which physiological coping mechanisms are exhausted and loss of water recycling due to increased plasma osmolarity (dehydration) has been discussed. The reader should refer to the chapters on Clinical Pathology and Anatomy and Physiology for an understanding of chelonian fluid management, and this information should be used in parallel with the advice and comments given below .

Aetiology

Excessively lengthy hibernation

In the wild, most specimens will have a long period of warm weather to prepare for a short hibernation period. In captivity, animals may be exposed to a short period of warm weather to prepare for a notably long period of dormancy, inactivity and hibernation. It may not be possible for its metabolism to adapt to this.

This author advises restricting hibernation in captivity to a maximum of 3 months regardless of size. Juvenile animals may be hibernated for as little as a month.

Leucopaenia

Leucopaenia is common following long periods of hibernation. If the tortoise was poorly maintained during the previous season, its white blood cell count may have been very low before hibernation was even begun. The life expectancy of white blood cells is limited, and where hibernation is prolonged during which time there is no regeneration of white blood cells, numbers may decline to extremely low levels. Such leucopaenic animals awake with compromised immune systems.

When leucopaenic tortoises are warmed (naturally or artificially) bacteria, mycotic and viral agents within them appear to replicate quickly, potentially before rebound and replication of white blood cell stem cells has been possible. In such circumstance stomatitis, rhinitis and systemic infections are common.

This scenario appears to be exacerbated by failing to allow a tortoise to reach its preferred body temperature rapidly following awakening, and allowing it to chill below its ATR at night, compromising the immune system but favouring the pathogens.

Inadequate post-hibernation husbandry

Inadequate care and lack of understanding of the physiological needs of chelonians are the main reasons for illness in the post-hibernation period in animals presented to this author (SM).

Common factors include:

- failure to observe that a confined animal is no longer in hibernation—sometimes for several weeks;
- failure to hydrate the animal—most animals will have been recycling bladder fluid without taking fresh fluid on board for about six months;
- failure to feed the animal. If a chelonian is maintained without suitable food or without the light and heat conditions necessary to allow it to eat, it will become catabolic. Many chelonians will have gathered inadequate energy reserves pre-hibernation, and warming the animal moderately without allowing food or fluid intake will result in muscle wastage and hypertonic dehydration.
- failure to provide suitable heat and light.

Disease or trauma during hibernation

Protection and observation are sometimes inadequate, resulting in:

- frost damage—the result of exposure to subzero temperatures. Thermal trauma to the central nervous system, eyes and frostbite to the distal limbs are the usual consequences;
- rat bites—common where tortoises are hibernated in cardboard boxes in outhouses and are too cold to escape from their predators.

Undetected chronic disease

Often the disease has been present, but asymptomatic, for a long period. All animals exhibiting inactivity or anorexia in the post-hibernation period should undergo a comprehensive examination, and the history should be thoroughly reviewed for predisposing adverse husbandry practices. Examples of such chronic disease conditions are:

- bacterial and mycotic infections;
- viral infections;
- lower respiratory tract disease;
- nutritional disease;
- dehydration;
- renal failure;
- follicular stasis/egg retention;
- gastrointestinal disease;
- hepatic disease;
- sight impairment;
- central nervous system disease;
- inappropriate environmental;
- inappropriate food provision;

- pain;
- maladaptation;
- social disruption.

History

History data (hibernation preparations, duration, monitoring, frequency of urination/defecation, post-hibernation vivarium conditions, appetite, food offered) are an essential part of the evaluation of the ill patient post-hibernation. Most healthy animals eat, drink and urinate within one week of emerging from hibernation. The reader is encouraged to consider underlying dehydration (presented in most *Testudo* spp. as hyperuricaemia, hyperkalaemia and anuria) in parallel with the management of other conditions and lesions.

Detailed advice on effective history taking is given in the section dealing with Assessment of Health. Some simple information should always be requested from the client, and the following are examples:

- What progress would this tortoise have made in previous years at this time of year?
- What are the animal's environment and climatic conditions? Does the client have a vivarium? How do they provide heat and light?
- What are the tortoise's day and night temperatures?
- Is the tortoise displaying abnormal behaviour e.g. respiratory movements?
- Is the tortoise displaying any specific clinical signs associated with disease, such as a nasal discharge?
- What is the condition of other tortoises owned by the client? How many are there? Have there been any recent introductions to the group, and were these quarantined?
- What is the quality of the client's normal husbandry?
- What nutrition is usually provided by the client?
- What provision has been made to ensure effective calcium metabolism?
- What health checks are performed by the client and how frequent are they throughout the year?

Clinical evaluation

An initial pre-treatment biochemistry assessment is an essential part of evaluating the PHA patient. A general tortoise profile at this author's surgery (SM) includes haematology (including differential white cell count), and examination for haemoparasites, total protein, potassium, uric acid, albumin, alkaline phosphatase, urea, calcium (ionised and total), GLDH, LDH, β-hydroxybutyrate, phosphate, AST, sodium, CK, and glucose. A base-line profile on admission allows sequential blood monitoring during hospitalisation and recovery. Following the results of a general profile, it is normal to monitor critical parameters such as PCV, albumin, uric acid, urea, and potassium throughout the stabilisation period. Through these parameters the response to fluids, nutrition and medication, such as allopurinol, can be assessed.

Urine acidity, urine specific gravity and blood β-hydroxybutyrate (BHB) appear to be additional parameters worth monitoring when stabilising a dehydrated terrestrial chelonian. In the immediate post-hibernation period of herbivorous chelonians it was found that pH was 5.0 and 6.0 but this rose to 8.0 and 8.5 after one month of normal feeding (Innis 1997b). Acidic urine (< pH 7) is suggestive of catabolism and is a consistent feature of herbivorous chelonians suffering from prolonged anorexia (Innis 1997b; JM/RJW: personal observation).

Urine specific gravity is likely to provide a sensitive indication of hydration status (Gibbons 2000), especially in uricotelic species, and particularly when uraemic, although this deserves further investigation. β-hydroxybutyrate (BHB) is thought to be a good indicator of ketogenesis in reptiles (Christopher *et al.* 1994). These authors found that, in desert tortoises (*Gopherus agassizii*), plasma levels varied from 0.4–0.75 mmol/l in times of significant rainfall (and food availability) but increased to 2.0 mmol/l after two months of drought. In contrast, ketogenesis did not appear to be important during hibernation.

Treatment

In animals where blood uric acid and potassium levels and historical urine output following hibernation encourage the clinician to feel that further treatment can be attempted, and euthanasia considerations postponed, the primary aims of treatment should be:

- To correct dehydration.
- To achieve diuresis and consequently decrease the elevated blood uric acid, potassium and urea levels. This generally involves fluid administration in combination with medication, such as with allopurinol.
- To elevate the blood glucose levels temporarily once diuresis and dehydration have been achieved. This will help reduce further catabolism adding to the azotaemia, and is normally achieved by adding appropriate nutritional products to the oral fluids administered.
- To provide optimal environmental conditions—we suggest hospitalisation in a vivarium.
- To provide appropriate nutrition, initially by hand feeding or via stomach-tube/oesophagostomy feeding, and then by reverting to the normal diet and withdrawing supportive feeding as self-feeding commences.
- To treat all concurrent disease or nutritional disorders effectively.
- To continue active fluid therapy and management of hyperuricaemia and gout for several weeks beyond recovery, as determined by observation.
- To improve the long-term care and management of the affected animal following initial recovery.

Fluid therapy

In hyperuricaemic, hyperkalaemic, hypoglycaemic post-hibernation *Testudo* spp., urgent fluid therapy (possibly in conjunction with drugs inhibiting further uric acid production) is essential to preserve life. This will help preserve renal function, maintain plasma osmolarity, prevent hyperuricaemia and gout, and increase the renal excretion and general metabolism of toxins, anaesthetics and medications. Fluid therapy may involve the use of orogastric tubes, oesophagostomy tubes and intracoelomic, intraosseous, intravenous, intracystic (bladder) or epicoelomic routes.

In hospitalised terrestrial chelonians treated at the author's surgery (SM) over the past three years, fluid therapy protocols

aim to restore urine output in anuric post-hibernation patients. In hyperuricaemic, hyperkalaemic cases presented to the author, it would appear safe to give fluids by the intraosseous, oral or epicoelomic routes at up to 4 ml/100 g/day, until multiple urination is achieved. Following this, fluid levels are generally reduced to 2 ml/100 g/day by oesophagostomy or stomach tube, as uric acid and blood potassium levels fall. Maintenance is continued for several weeks at least at about 0.5–1 ml/100 g/day.

The intraosseous and epicoelomic routes are of help during early management, especially where uric acid levels are above 16.81 mg/dl (1000 µmol/l), but they must be used with caution, as it is impossible to quantify how much fluid is required. Excessive hydration by these routes, especially in the absence of renal output, may cause pulmonary or cerebral oedema. Generally, the author's routes of choice are oral fluids over time, soaks, and cloacal/bladder lavage and irrigation. In the author's opinion, intravenous fluid therapy is really an emergency life-saving measure and of limited benefit to a chronically ill hyperuricaemic patient. Other clinicians, however, anecdotally report intravenous fluid therapy to be of use.

Hospitalisation

Hospitalisation protocol and record keeping are an essential part of the evaluation of recovery. Without admission into a therapeutic hospitalisation environment, it is hard to gauge the impact of any treatment.

With respect to dehydration, this author would advocate the hospitalisation of *Testudo* species with the following signs or biochemistry markers:

- hyperuricaemia (uric acid > 11.76 mg/dl, > 500 µmol/l);
- hyperkalaemia (k^+ > 19.5 mg/dl, > 5 mmol/l);
- anuria (no urine within the previous 10 days).

These animals are generally hospitalised on a fluid therapy protocol as described below until repeated urination, decreasing uric acid levels and decreasing potassium levels are achieved. This author has found that many dehydrated terrestrial *Testudo* spp. can be stabilised satisfactorily providing that severe renal pathology does not exist.

Prognosis

Animals with uric acid levels above 33.61 mg/dl (2000 µmol/l) and potassium levels above ~35 mg/dl (9 mmol/l) often demonstrate no urine output despite active fluid therapy. Death in such animals appears to be the result of hyperkalaemic cardioplegia (heart failure) unless animals are euthanased. Histopathology in five recent terminal hyperuricaemic cases suggests that renal failure is due to loss of functional renal tubules because of active urate excretion in the proximal tubes, without sufficient glomerular filtrate to carry this urate sludge away. Glomeruli are often ruptured and contain excessive crystalline urate precipitates (SM: personal observation).

Lawrence (1987) suggests that it is unrewarding to treat post-hibernation anorexia in *Testudo* cases demonstrating a blood urea of more than 560 mg/dl (200 mmol/l), but that tortoises with a post-hibernation urea level of 420 mg/dl (150 mmol/l) often respond well to treatment. As he did not assess potassium or uric acid values it is possible that his figures reflect a combination of catabolism and dehydration. This author would advocate the assessment of uric acid and potassium values wherever possible.

Continued supportive care

Following initial urination, nutritional support should be offered. Donoghue (1996) proposes that the treatment of energy deficiency in chelonians should first involve fluid and electrolyte replacement and then small but increasing levels of calories and nutrients in order to reduce the possibility of re-feeding syndrome. However, in hyperkalaemic patients, it is advantageous to give glucose to drive potassium ions into the cells. This author uses Critical Care Formula® (Vetark, UK), initially double diluted. This means half the recommended feeding amount, is given in order to counter re-feeding syndrome. It is wise to monitor blood potassium levels, urine output, uric acid levels, and activity throughout this period. Alternatively, the normal diet is liquidised and blended with dilute Critical Care Formula and powdered fibre.

Medication

Allopurinol (Allopurinol BP, Generics UK, Potters Bar) is a standard medication given by this author to all chronically ill chelonians with uric acid levels above 16.81 mg/dl (1000 µmol/l). Generally, allopurinol is given at 20 mg/kg/day by dissolving a 100 mg tablet in 5 ml of water and then offering 1 ml/kg/day PO, or by stomach/oesophagostomy tube. In most cases, treatment is continued for about three months, with further therapy dictated by regular blood biochemistry assessment. Correction of husbandry problems, especially those associated with possible nutritional hyperparathyroidism, may reduce the long-term need for allopurinol, which, however, seems to be very well tolerated.

This author has not found probenecid (Benemid®, MSD) to be of major use in these cases. All animals treated with probenecid by the author have died, and histopathology of cases has suggested that increased active excretion in the absence of a glomerular filtrate has resulted in irreparable glomerular and tubular damage.

Bladder lavage

Bladder lavage, involving the cloacal insertion of a Foley catheter into the bladder and lavage of its contents (Dantzler & Schmidt-Nielson 1966) (Fig. 10.38), offers exciting possibilities for the future stabilisation of hyperuricaemic, hyperkalaemic patients. Bearing in mind the function of the lower urinary tract, it should be possible to remove excess potassium and uric acid, and to administer fluids (and possibly even medications such as allopurinol) by this route. Initial trials at the author's surgery using water have been interesting, but it is too early to draw any firm conclusions.

Euthanasia

Euthanasia is the treatment of choice for post-hibernation anorexia cases that are considered to be beyond recovery. However, identification of these cases is generally based upon a failure to respond to stabilising fluid therapy.

We have successfully treated cases where uric acid had risen to 30.25 mg/dl (1800 µmol/l), but never a case exceeding 33.61 mg/dl (2000 µmol/l). Similarly, blood potassium of 26.92 mg/dl (7 mmol/l) or less might be stabilised whereas potassium of 34.62 mg/dl (9 mmol/l) or more has been consistently fatal.

In the author's experience, renal biopsy does result in an understanding of renal pathology, but has not yet been able to differentiate cases capable of recovery from those that are beyond help. Cases that fail to urinate, but where appropriate hospitalisation and fluid therapy is given as described above (i.e. greater than 2 ml/100 g/day by any combination of routes) over a period of ten days, are candidates for humane euthanasia. Euthanasia technique is described in detail elsewhere.

RENAL DISEASE

Aetiology

Jacobson (1994) uncovered relatively few reports of renal disease in chelonians. Most reports are based upon necropsy surveys. Keymer (1978a & b) found 4.9% of 144 terrestrial tortoise necropsy examinations to have evidence of nephropathy and 11.5% of 122 freshwater and marine turtle necropsy examinations to have evidence of nephropathy. The lack of literature describing renal failure and its causes is a consequence of our limited ability to diagnose renal disease ante-mortem. It does not mean that the prevalence of chelonian disease is low.

Dehydration

In chronically ill chelonians, those receiving medication or those undergoing anaesthesia, it is essential to maintain adequate fluid input. This will help preserve renal function, maintain plasma osmolarity, prevent hyperuricaemia and gout and increase the excretion and metabolism of renally excreted toxins, anaesthetics and medications. Fluid therapy may involve the use of stomach tubes, oesophagostomy tubes, intracoelomic fluids, intraosseous fluids, intravenous fluids and the epicoelomic route.

Cooper & Jackson (1981) point out that reptiles dying of starvation and dehydration often have renal tubules that are completely plugged with crystalline material, resulting in renal tubular blockage. Active excretion of urates during dehydration results in intrarenal renal failure. This is because *in vivo* dissolution of urate precipitates with rehydration therapy is not readily achievable, consequent upon their low water solubility. It is therefore this author's opinion (SM) that probenecid therapy should be avoided in dehydrated reptiles, as it may exacerbate renal tubular blockage.

Parasites

Zwart & Truyens (1975) describe *Hexamita parva* infection. This has now been reported in species of *Testudo, Geochelone, Cuora, Terrapene, Geoemyda* and *Clemmys*, and can result in a life-threatening nephritis. It is necessary to differentiate *Hexamita parva* from intestinal trichomonads, as their presence in faecal and urine samples may be normal.

Drugs

Various drugs are nephrotoxic. This is especially true of the aminoglycosides (gentamicin, amikacin and kanamycin). Frye (1991a) suggests avoiding the use of these agents in reptiles generally. It is wise if they are only selected where sensitivity results indicate they are the antimicrobial of choice.

Viruses

There are no reports of viral diseases that result in purely renal pathology. However, kidney pathology is possible in combination with other systemic pathology. Müller *et al.* (1990) investigated an outbreak of herpes-virus-associated disease where inclusion bodies were observed in glomerular tissues. The authors suggested renal excretion and horizontal transmission of this virus might occur. McArthur (2001) described lymphoid infiltration of many organs, including liver, spleen, lung and kidneys, in the presence of herpes-virus-like particles.

Bacteria

Any organism isolated from a renal infection is likely to be present in other areas of the body as well. Disseminated infections like this are likely to occur during chronic nutritional disease or viral disease, although a discrete granuloma or an abscess/fibriscess within renal tissue is also possible. Various bacterial and fungal organisms may be isolated in pyelonephritis, but none are specific to renal disease. The reptilian renal portal system (Holz *et al.* 1994; Holz 1999) may allow infections that establish in the chelonian tail to spread haematogenously to the kidneys. Pyelonephritis may also result from ascending urinary tract infections (Frye 1991a).

Diet

Anecdotal evidence suggests that some diets may precipitate renal failure in reptiles. Diets with excessively high purine and protein levels or significant animal protein are inappropriate to herbivorous chelonians. High purine levels will predispose to hyperuricaemia in any reptile and are best avoided wherever possible.

Hyperparathyroidism

Accelerated growth, soft shell, and other manifestations of metabolic bone disease in adolescent chelonians appear anecdotally associated with renal failure (SM: personal observation). Hyperparathyroidism appears to predispose to renal disease (Slatopolosky *et al.* 1980; Endlich *et al.* 1995; Nami & Gennari 1995; Rosol *et al.* 1995; Nagode *et al.* 1996).

Clinical signs

There are no clinical signs specific to renal disease.

Behavioural changes

These are non-specific and include inactivity, weakness, malaise, debility and anorexia.

Changes in urine output

Renal disease may be characterised by changes in urine output (anuria, oliguria or polyuria). Clinically, such changes are hard or impossible to detect, as some urine from the urodeum is mixed in the proctodeum and colon and appears with faeces. In addition, the frequency with which a tortoise voids its urine is dependent upon external factors such as drinking, dietary water content, environmental humidity and bathing.

Dehydration

Animals with pre-renal azotaemia (hyperuricaemia/uraemia) may occasionally show clinical signs of dehydration (reduced

skin elasticity, decreased urine output, sunken eyes). Dehydration is not specific to renal disease and is not an invariable finding.

Generalised oedema

Iatrogenic over-hydration often occurs in animals being given fluid therapy during renal disease. This is common in dehydrated uricotelic species where renal tubules and glomeruli have become obstructed by urate deposits.

Hypoproteinaemia may result from protein-losing nephropathy and this may also cause oedema.

History

Most features of the history will be non-specific and animals are often presented following prolonged periods of inactivity, anorexia and anuria, which may result from almost any husbandry/nutritional related or infectious disease. Specific features of the history of interest include:

- Nephrotoxins—
  (1) administration of nephrotoxic medications;
  (2) exposure to possible natural nephrotoxins (e.g. toxic plant ingestion or access to poor quality/contaminated sources of water).
- Dehydration—inadequate fluid intake or failure to provide an adequate water source. Dehydration is likely to precipitate renal disease and exacerbate any existing renal problems.
- Inappropriate hibernation management—inappropriate hibernation and/or post-hibernation management.
- Nutrition—high protein diets, excessive vitamin D_3 supplementation, poor vitamin A provision.
- Infectious agents—exposure to animals harbouring infectious agents.

Diagnosis

There are both physiological and practical reasons why it has not been possible to develop anything other than crude assays of chelonian renal function. Methods such as clearance ratios used in mammalian medicine are not applicable because of lower urinary tract changes in urine composition.

Ante-mortem diagnosis of renal disease is complex, and often depends upon associating various findings.

The diagnosis of pyelonephritis in a living tortoise is unusual without the aid of endoscopic biopsy or as an incidental finding made at coeliotomy. However, renal casts and a heterophilia, with or without monocytosis or azurophilia may be present, and variable elevations in AST, LDH, uric acid and potassium may occur. Pyelonephritis is more commonly diagnosed through histopathology of post-mortem samples.

The diagnosis of hexamitiasis in a living chelonian is complex, because trichomonads may be present in urine from normal animals and renal biopsy associating the protozoan with pathology is necessary. Haematological and urine microscopy findings can only be suggestive.

Blood biochemistry

No single biochemical criterion can be relied upon as an unequivocal measure of renal function. Combinations of measured parameters appear to offer little improvement in sensitivity and specificity in the assessment of renal disease. Blood levels of urea, uric acid, creatinine, enzymes (AST/LHD), albumin, electrolytes including potassium, calcium and phosphate and ratios of blood calcium to inorganic phosphate have been cited in the literature to assess renal disease in reptiles. However, in chelonians no such variations relate purely to renal disease. Various parameters are described briefly below in relation to renal disease observed by this author (SM). Further comment on blood biochemistry assessments can be found in the Clinical Pathology section of this book.

Uric acid

Uric acid elevation (hyperuricaemia) occurs when inadequate amounts of uric acid are excreted, or too much is produced. Hyperuricaemia is not a reliable measure of renal disease, as it will also rise if dehydration decreases the glomerular filtration rate. Excessive dietary protein can lead to high serum uric acid. Hepatopathies and lymphodilution may depress blood uric acid levels. Distinguishing pre-renal hyperuricaemia (dehydration, dietary induced) from renal hyperuricaemia is not straightforward and may require renal biopsy and histopathology. Values in healthy chelonians vary greatly, and published ranges are not available for most species, especially with respect to season, sex and environmental conditions.

This author (SM) considers values less than 250 µmol/l to be normal in Mediterranean tortoises during summer, when they are at their lowest. Values in tortoises considered to be in good health are often less than 100 µmol/l. Plasma levels of 350 µmol/l or more suggest that the kidneys are not excreting uric acid effectively, either because of dehydration or renal disease. Values of up to 350 µmol/l can be normal in the immediate post-hibernation period. Values above 350 µmol/l in the immediate post-hibernation period suggest that the tortoise has relied upon protein catabolism, possibly because of unsuitable temperatures or inadequate carbohydrate reserves during hibernation, or that the animal is dehydrated. Such animals require veterinary intervention.

In Mediterranean tortoises, sustained elevations of uric acid, to levels of 1000 µmol/l or more, may occur in life-threatening renal disease and dehydration, regardless of season. Where plasma levels exceed 1500 µmol/l then urate crystal deposition (gouty tophi) may occur in healthy tissue (Zwart 1992). However, tissues may suffer from gout deposition at significantly lower uric acid levels because of factors that trigger off the crystallisation of uric acid (high plasma osmolarity, tissue inflammation, the presence of uric acid reactive cations). Prolonged fluid therapy to dissolve visceral gouty tophi, in combination with medications such as allopurinol, will be necessary in such cases if animals are to survive.

Tortoises with sustained uric acid levels above 2000 µmol/l (33.61 mg/dl) are extremely hard to stabilise. Where uric acid levels continue to remain above 2000 µmol/l despite several days' therapy, euthanasia should be considered. Often uricotelic species with values above 2000 µmol/l remain anuric despite fluid therapy.

Urea

Blood urea values are of limited value when assessing reptilian renal function, as urea is highly variable in both production and excretion, with levels often raised, in the absence of renal pathology, during dehydration. Chelonian species vary in their method

of nitrogenous excretion, with some species being predominantly aminoureotelic, some predominantly uricotelic and others ureouricotelic. This has a significant effect on whether urea, uric acid or both become elevated during renal failure and dehydration. Elevations in blood urea values are the result of dehydration, catabolism, renal disease or any combination of these factors.

In *Testudo* spp. presented to this author (SM), blood urea levels above 20 mmol/l are often present with chronic starvation, dehydration and/or renal disease. With severe dehydration, blood urea levels can rise to 100–200 mmol/l. Lawrence (1987b) suggests that it is unrewarding to treat cases demonstrating a blood urea of more than 200 mmol/l, but that tortoises with a urea level of 150 mmol/l respond well to treatment.

Creatinine

Variations in both production and excretion of creatinine in most reptiles limit interpretation of plasma levels in relation to pathology. Assay methodology may also have significant effects.

Hyperuricaemic tortoises presented at this author's (SM) surgery occasionally demonstrate moderately elevated creatinine values, but it is unknown whether these are the result of dehydration, renal disease, both or some other cause.

Most sick chelonians presented to the author have levels below our laboratory's reference range, regardless of other indicators of renal function.

Albumin

We have found hypoalbuminaemia in tortoises with end stage renal disease, but this is obviously a relatively non-specific finding. Hypoalbuminaemia may occur in cases of glomerular disease due to excessive renal losses, however low albumin values can also reflect hepatopathies, protein-losing enteropathies and periods of starvation. Albumin levels become elevated in female tortoises in vitellogenesis (usually during summer) and this will affect the interpretation of blood protein levels. Elevations related to vitellogenesis should be expected in association with high blood calcium levels.

Potassium

Hyperkalaemia (a potassium value greater than about 5.5 mmol/l) appears to be common in dehydrated *Testudo* spp. presented to this author (SM). Hyperkalaemia is possibly the result of reduced glomerular filtration and a failure of fluid to enter the bladder, resulting in decreased excretion at bladder level. Elevated plasma potassium levels may also be the result of severe tissue damage, and haemolysis of blood cells may result in falsely elevated values.

Values above 5 mmol/l should be actively treated with fluid therapy (as described in the section the post hibernation tortoise). Exposure to values above 9 mmol/l (34.62 mg/dl) seems to be consistently fatal. This is presumably due to depressive effects upon heart muscle.

Calcium

The levels of blood calcium in higher vertebrates in renal failure are highly variable, and hypocalcaemia, normocalcaemia and hypercalcaemia are all possible (Feldman 1995). This also appears to be the case in chelonians. However, chelonian blood calcium levels are affected by a variety of *in vivo* and *in vitro* factors, which may mask any variations caused by renal function (folliculogenesis, excessive supplementation, hypervitaminosis D, lipaemia, albumin concentration, lymphodilution and haemolysis).

Calcium levels in Mediterranean tortoises with end-stage renal disease seen at this author's (SM) surgery have ranged from 1.78–4.26 mmol/l. No specific trend has been apparent.

Inorganic phosphate

Inorganic phosphate is significantly affected by husbandry, nutrition and vitamin/mineral supplementation in captive chelonians. Parathyroid hormone maintains blood calcium levels at the expense of blood phosphate levels. These influences may mask any changes due to renal function.

Inorganic phosphate levels were in the range 1.48–1.91 mmol/l in cases where kidneys were end stage (SM). It is possible that variations in blood phosphate levels during renal disease are more subtle in chelonians, than in other reptiles.

Calcium:inorganic phosphate ratio (Ca:PO_4)

The Ca:PO_4 ratio is affected by the individual factors influencing plasma calcium and inorganic phosphate levels. These include husbandry, general nutrition, vitamin and mineral supplementation, age, season, sex and renal disease.

The mean September ratio (mg/dl values) in 12 healthy, captive Mediterranean tortoises was 3.33:1. The mean for six females was 3.12:1 and for six males 3.54:1 (Harcourt-Brown 1998: personal communication).

It is suggested that a Ca:P ratio less than 1:1 mg/dl occurs during renal disease in *Iguana iguana* (Boyer 1996aa; Campbell 1996; Mader 1997; Divers 1998a). The Ca:P ratio (using mg/dl) of tortoises at our surgery with end stage renal disease has been 1.20–3.71:1. We have not yet seen a chelonian Ca:P ratio less than 1.

Elevations in inorganic phosphate levels in relation to calcium might be expected during renal failure but it is not known to what extent such alterations occur in chelonians. The Ca:P ratio in tortoises in renal failure requires further investigation, as we have very limited data. It may yet prove to be a sensitive measure of renal function, but the magnitude of change may differ from that seen in *Iguana iguana*.

Solubility index (Ca [mmol/l] × PO_4 [mmol/l])

Hyperuricaemic tortoises presented to this author (SM) appear to have a raised solubility index (average 4.42), compared to normal tortoises (average 2.54). They do not need to reach the values suggested by Divers (1998a) to result in soft tissue mineralisation (9). It is possible that depressed phosphate levels occur more often in United Kingdom captive tortoises when compared to other reptiles. The highest values observed in clinically dehydrated, terminal, hyperuricaemic cases were in the region of 7.48.

Renal enzymes

Lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) have been found in reptilian kidney tissue by Ramsay & Dotson (1995). However, because these enzymes are not tissue specific and may vary with species, interpretation of any elevations of plasma enzyme level is complex and investigations into isoenzyme analysis are needed.

In cases of chronic renal failure presented to this author (SM), both LDH and AST values have been consistently elevated. The

magnitude of LDH and AST alterations does appear to relate to the level of uric acid elevations.

Mean LDH and AST for tortoises with end-stage renal disease were 3093 IU/l (LDH) and 297 IU/l (AST). Levels of other enzymes, such as GGT and AlkP (alkaline phosphatase), appear to have remained normal. Average values calculated for 12 normal captive *Testudo* in September, were 165.1 IU/l (LDH) and 66.4 IU/l (AST) (Harcourt-Brown 1994: personal communication).

Renal function tests

These are not yet available.

Urine

Urine does not help in the assessment of renal function of chelonians in the same way it does in mammals, but monitoring certain aspects of urine can be helpful in determining renal status and suggesting the potential for renal disease.

Monitoring output

Monitoring the frequency of urination of any chelonian is important and should be detailed when recording the client history, and during the hospitalisation of any debilitated chelonian. Significant dehydration will result in decreased urine production, and this leads to concentration of nephrotoxins. Factors influencing urination in tortoises are poorly understood. It would appear that events such as urination and bathing/access to fluids are related.

In most hospitalised chelonians at this author's (SM) surgery, we have found adequate fluid administration results in urination every second day. Animals are bathed daily and given 0.5%–2% of body weight as fluids per day, in divided doses. Where fluid output decreases beyond once every five days, despite active fluid therapy, and marked fluid retention occurs, then renal disease (especially tubular tophi) may be present.

Urine specific gravity and pH

Urine specific gravity and acidity may be sensitive measures of hydration status and catabolism in herbivorous uricotelic chelonians. By monitoring such parameters it may be possible to identify chelonians at risk of hyperuricaemia because of decreased glomerular perfusion.

In the immediate post-hibernation period of herbivorous chelonians it was found that pH was 5.0 and 6.0, but increased to 8.0 and 8.5 after one month of normal feeding (Innis 1997).

Acidic urine (<pH7) is indicative of protein and/or fat catabolism (ketones) and is a consistent feature of herbivorous chelonians suffering from prolonged anorexia (Innis 1997; JM/RJW: personal observation). Urine specific gravity is likely to provide a sensitive indication of hydration status (Gibbons 2000), especially in uricotelic species, especially when uraemic, although this would benefit from further investigation

Microscopy and cytology

Renal casts containing bacteria or fungi may be noted during microscopy of urine—suggesting pyelonephritis. Reptile urine, even if collected by cystocentesis, is not normally either sterile or of a predictable concentration. Osmotic and electrolyte changes occur in the bladder due to post-renal fluid reabsorption, electrolyte exchange and urate precipitation. The active absorption of bladder fluid in dehydrated and hibernating tortoises is discussed in Lawrence (1987a) and Dantzler & Schmidt Nielson (1966). Whilst this may be helpful to the tortoise it limits the information that can be gained about renal function from bladder samples.

Urine microscopy and cytology may demonstrate erythrocytes, leucocytes, and other inflammatory cells. Renal casts may be indicative of tubulonephrosis/pyelonephritis. Parasites such as *Hexamita* may be apparent and bacterial and fungal pathogens may be cultured. Care must be taken when interpreting cytology and culture findings from this non-sterile site, as the normal flora of the chelonian bladder has not yet been established in any species.

Diagnostic imaging techniques

Diagnostic imaging techniques, such as radiography, endoscopy, MRI or ultrasonography, may suggest renal gout or other lesions/pathology. Pyelonephritis may cause a significant enlargement of an affected kidney and Frye (1991a) suggests this may even mistakenly give the impression of neoplasia.

Biopsy

As already discussed, accurate biochemical assessment of renal function is not yet possible in chelonians, particularly without catheterisation of the ureters. Most renal conditions, such as tubulonephrosis, nephritis, nephrocalcinosis, glomerulonephritis, hexamitiasis, amyloidosis and neoplasia will require renal biopsy or necropsy and histopathology or electron microscopy to achieve a diagnosis.

Unfortunately, the indications for early renal biopsy in chelonians are not well established. Samples collected once disease is advanced are likely to demonstrate similar histopathology, regardless of the cause.

Renal biopsy may be carried out during a coeliotomy. This may be through a plastron osteotomy or endoscopically through an approach anterior to the hind limb. Chelonian anatomy can restrict the ability to perform renal biopsy. Where the above techniques are impractical it is also possible to burrow retrocoelomically anterior to a hind limb and reach renal tissue for biopsy. Here care must be taken to limit damage to vascular and nerve structures present in the area.

Complications such as deterioration in renal function, obstruction of ureters with blood clots and haemorrhage must be considered.

Treatment

Effective treatment of compromised renal function and renal failure involves fluid therapy in combination with management of any specific underlying cause. Management of gout, hyperparathyroidism and post-hibernation hyperuricaemia, hyperkalaemia and anuria are also described separately in this book.

Giving fluids, energy provision and supportive nursing are more important than trying to feed a normal diet during the initial stabilisation of chelonians with renal disease. During later recovery, and following stabilisation, diets restricted in purines and of high biological availability, with energy provision through simple fats and carbohydrates, are suitable. This author (SM) currently uses Critical Care® (Vetark, UK).

It is important to maintain gut flora in severely debilitated, chronically anorectic, herbivorous species. A liquidised form of

the normal diet and Avipro® (Vetark, UK) are used at our surgery, with the focus initially on rehydration with fluids of moderate energy content and low but high-quality protein and amino acids. Allopurinol and anabolic steroids also appear to help.

Fluid therapy

All cases, unless exhibiting signs of over-hydration, benefit from fluid therapy to maintain a plasma osmolarity at a level where glomerular filtration can occur, particularly where an inadequate fluid intake is likely. During hypertonic dehydration, as in hyperuricaemia associated with pre-renal azotaemia, fluid should be hypotonic in order to counter increased plasma osmolarity.

Fluid can be administered by oral, intravenous, coelomic, epicoelomic and cloacal/bladder routes. Suggested rates of administration are given earlier in the Hospitalisation section of this book.

Oral water at 0.5%–3% body weight per day (in divided doses) is ideal. Higher rates are appropriate during initial stabilisation, until urination is confirmed more than once, lower rates can be used thereafter for maintenance. Bath tortoises frequently, once or twice daily in order to encourage drinking, urination and encourage cloacal fluid uptake.

The epicoelomic route is ideal in acute dehydration. Alternatively, the intraosseous route can be used with a syringe driver or paediatric (burette) giving set. The oral route can be used subsequently, once the patient has been stabilised.

The use of an oesophagostomy tube is indicated in cases where the need for oral medication is likely to be chronic, and where handling is likely to traumatise the patient, or where the patient is recalcitrant.

Fluid retention and subcutaneous oedema in hyperuricaemic tortoises, in the absence of hypoalbuminaemia or an obvious cause of cardiac insufficiency, is an indication that fluid administration should be ceased. Some cases are best euthanased if in distress. Weight and urination should be monitored carefully in combination with blood parameters such as potassium and uric acid.

Reduction of hyperuricaemia

Hyperuricaemia is an indication for immediate fluid therapy, possibly in conjunction with drugs that decrease uric acid manufacture and increase its excretion. Where hyperuricaemia is present, the patient is at risk from precipitation of uric acid complexes within viscera. Medications to moderate hyperuricaemia are also discussed in the section describing gout. Fluid techniques have already been described above.

Allopurinol

Allopurinol decreases hepatic formation of uric acid through inhibition of xanthine oxidase. It seems ideally suited to the long-term management of hyperuricaemia in uricotelic chelonians. Allopurinol is used by this author (SM) in uricotelic species with blood uric acid values above 500 µmol/l in order to reduce the risk of visceral gout. Chelonians have received this product from this author over a period of several years without adverse effect.

Kölle (2001) found that a dose of 50 mg/kg/day suited most hyperuricaemic *Testudo*. In her experience, inappetence, inactivity and depression improved three to four weeks after commencement of treatment. Renal deposits of uric acid monitored using ultrasonography took two to four months to clear. Long-term therapy (six or more months) was advised. Further doses from the literature are also described in the section describing Gout.

Probenecid

It has been proposed that the action of probenecid is to increase tubular excretion of uric acid, and to decrease tubular reabsorption of uric acid. However, this author has not experienced anything other than dramatic deterioration in condition and blood uric acid values in hyperuricaemic uricotelic species using this drug. This appears to be related to increased tubular excretion of uric acid in the absence of sufficient glomerular filtrate to carry it away.

Nephrotoxic medications

Dehydrated chelonians, or cases with suspected renal disease, should never be medicated with potentially nephrotoxic drugs. Medications containing urate-reactive cations should be administered cautiously in such animals and preferably not at all, or urate salts may be inadvertently precipitated out of solution resulting in gout (examples include calcium and potassium salts precipitated by enrofloxacin injections).

Systemic aminoglycosides are best avoided unless culture and sensitivity results suggest them to be the antimicrobial of choice. All non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided where the patient is dehydrated or suspected to be suffering from renal disease.

Systemic antibiotics

Treatment of pyelonephritis with antibiotics or antifungal agents will depend upon the identity and sensitivity pattern of the organism responsible, as well as cost, safety, ease of administration and pharmacokinetics of medications. Antibiotics are justified where bacterial pyelonephritis is present or suspected from urine analysis, blood or other evaluations, or when concurrent bacterial disease is apparent.

Where immunosuppression is present or suspected (e.g. herpesvirus or chronic starvation/inappropriate nutrition) routine broad-spectrum antibiotic cover may be justified.

Systemic antimycotic agents

Antimycotic agents are justified where mycotic pyelonephritis is present or suspected from urine analysis, blood or other evaluations, or concurrent mycotic disease is apparent. Where immunosuppression is present or suspected (e.g. herpesvirus or chronic starvation/inappropriate nutrition), routine antimycotic cover may be justified.

Antiprotozoans

Where *Hexamita parva* or other protozoan pathogens are present or suspected the use of antiprotozoan drugs may be indicated. It is important to differentiate normal gut trichomonads from *Hexamita parva*. Antiprotozoan drugs such as metronidazole are discussed in the Therapeutics section of this book.

Environmental support

Optimise environmental provision by maintaining temperature, light intensity and humidity ideally suited to the species. Bath animals frequently, once or twice daily in order to encourage drinking, urination and encourage cloacal fluid uptake.

Nutritional support

Hand feeding, tube feeding, oesophagostomy and stomach tube feeding may all help rehabilitate the patient. Diets restricted in purines and excessive protein should be used. At this author's (SM) surgery, high-energy products such as Critical Care Formula® (Vetark, UK) are used in combination with fluids.

During the initial stabilisation of a catabolic, anorexic reptile with signs consistent with renal failure, administration of high-energy diets and fluids should be attempted. Glucose solutions are helpful once dehydration has been corrected in post-hibernation anorexic tortoises, and may help to reduce elevated blood potassium levels by driving potassium into cells.

The use of an oesophagostomy tube or stomach tube should be considered.

The type volume, frequency, and timing of foodstuffs will depend on the species and its condition. Examples are given earlier in the Hospitalisation section of this book. As a positive response to treatment occurs the animal is gradually weaned onto its normal diet in liquidised form, in combination with the opportunity to self-feed.

Monitor urine output

This is discussed earlier. Interpretation in some debilitated species may be clouded by behavioural influences, voiding of ureteral fluid with faeces and bladder equilibration factors (Dantzler & Schmidt Nielson 1966).

A uricotelic tortoise urinating frequently will detoxify by voiding bladder potassium and urates. Most hospitalised chelonians at the author's clinic urinate at least every second day if adequately maintained with suitable fluids (0.5%–3% body weight per day). A well-maintained tortoise urinating less than once every five days is unusual if bathed and given appropriate fluids, unless there are serious metabolic problems such as hyperuricaemic or hyperkalaemic renal failure.

Coelomic dialysis

Frye (1991) describes some success using this technique (Fig. 8.39). However, the procedure sacrifices coelomic protein and so must not be performed excessively. This author would advise that bladder lavage and fluid administration are more suitable in anuric, hyperkalaemic, hyperuricaemic cases.

Bladder lavage and fluid administration

Bladder lavage and fluid administration are relatively simple in chelonians >700 g, using a Foley catheter passed into the bladder through the cloaca (Fig. 8.38). A technique to pass a Foley catheter into the bladder of *Geochelone agassizii* is described by Dantzler & Schmidt Nielson (1966).

The catheter, once inserted, is available for voiding and lavage of bladder toxins, direct administration of hypotonic fluid into the bladder (fluid therapy) and potentially even drug administration (e.g. allopurinol).

Monitor blood biochemistry

Blood biochemistry parameters defining renal failure are still poorly understood, but in distressed cases, responding poorly to treatment, they may help justify humane euthanasia. This author (SM) would encourage clinicians to measure blood uric acid, urea and potassium levels throughout recovery, perhaps weekly, for hyperuricaemia in uricotelic species.

Euthanasia

Euthanasia should be considered where stabilisation has not been possible, despite appropriate fluid therapy and medication. Cases where profound fluid retention occurs, where blood uric acid levels are consistently above 2000 µmol/l (33.61 mg/dl) and with profound hyperkalaemia (>8.5 mmol/l) have a very guarded prognosis.

SEPTICAEMIA

(Figs 11.37, 11.86–11.87)

Aetiology

Septicaemia is common in any immunocompromised chelonian. Typically, cases occur in:

- the semi-aquatic chelonian immersed in poor quality water for long periods, where filtration methods are inadequate and haul-out areas are limited;
- a chelonian maintained below its ATR;
- animals maintained with poor hygiene;
- animals with untreated open wounds and lesions.

Bacteria involved are often Gram negative and enter through wounds or disseminate from ear abscesses etc.

Clinical signs

Septicaemia should be suspected wherever localised infections are apparent. Signs associated with septicaemia are relatively non-specific and include:

- acute debility;
- erythematous plastron flush;
- petechial haemorrhages on mucous membranes;
- sudden death.

History

Septicaemia is often secondary to chronic (possibly unnoticed) disease. Signs are generally non-specific but may include a sudden and dramatic decline in the health and demeanour of the patient. Animals may have obvious infections acting as the source of the septicaemia and the history may reveal that husbandry conditions may not have been ideal.

Diagnosis

Blood culture and/or cytology are necessary for identification of organisms causing septicaemia.

Haematology may reveal toxic heterophils, sometimes in the presence of bacteria. Such findings justify the instigation of rapid and aggressive therapy. However, with both culture and cytology techniques, contaminating organisms may be hard to distinguish from pathogens.

- Disseminated infections (joints, liver etc.) suggest previous septicaemia.
- Septicaemia may be a post-mortem finding.
- Septicaemia may become a presumptive retrospective diagnosis because of the response to antimicrobial treatment.

Treatment

Effective treatment of septicaemia is multifactorial. Husbandry and nutrition should be optimised and any primary disease, such as wounds and open lesions, should be treated.

Intravenous, intraosseous or intramuscular antimicrobial medications should be given, in combination with supportive fluid therapy and nutritional support in a hospitalisation environment. Antimicrobials should be chosen by culture and sensitivity if possible, otherwise use combinations as described later in the Therapeutics section of this book.

SIGHT PROBLEMS

(Figs 11.12–11.21)

Aetiology

Chronic debility of almost any sort appears to result in deterioration of vision in *Testudo* spp. Various causes are possible:

- keratitis/keratoconjunctivitis (chlamydial, bacterial, mycotic, mycoplasma, viral);
- corneal trauma;
- lipidosis;
- scar tissue;
- frost damage during hibernation;
- excessive exposure to ultraviolet light;
- hypovitaminosis A;
- metabolic disease;
- hepatopathy;
- CNS/visceral gout;
- possibly CNS viral infection.

Clinical signs

Some animals remain immobile and fail to eat, but display little evidence of the cause. Some animals are mobile if heated appropriately, but fail to stop when presented with solid objects or falls from heights. They may display poor visual reflexes (menace, pupillary light etc.) and/or ocular lesions may be apparent.

History

The history will be variable depending upon the aetiology. Often sight-impaired animals are referred by clinicians unable to determine a specific cause of inactivity and anorexia despite the good environmental and nutritional care. Concurrent disease is common.

Diagnosis

All inactive or anorexic animals deserve an ocular examination, a complete clinical examination and extensive work-up.

Treatment

- Remove any predisposing causes.
- Improve husbandry and nutrition.
- Establish a long-term nursing/rehabilitation protocol.
- Lawton & Stoakes (1989) suggest that frost-damaged tortoises may respond to long-term nursing in conjunction with long-term, low dose, oral vitamin A supplementation.

STEATITIS

(deficiency of vitamin E/selenium complex)

Aetiology

Steatitis occurs in many types of reptiles where an excessively fat-rich (or otherwise unsuitable) diet has been provided. A high fat diet is not suited to herbivorous species. In omnivorous and carnivorous species it should be used carefully, if at all.

Whilst this is an uncommon problem with chelonians, Frye (1991b) reports steatitis being recorded in aquatic chelonians because of a combination of vitamin E and selenium deficiency. Species such as the red-eared slider (*Pseudemys scripta elegans*) and other similar semi-aquatic omnivores would therefore appear to be susceptible. Frye (1991b) likens the condition to white muscle disease and proposes the cause as 'an anomalous, unnatural diet lacking in variety' often based around oily fish. Frye also proposes that forage grown in selenium-deficient areas and fed to herbivorous reptiles may also result in steatitis.

Clinical signs

Clinical signs associated with steatitis are suggested by Frye (1991b) to include grossly abnormal fatty tissue that is unusually firm on palpation. The overlying skin may also be discoloured yellow.

Treatment

Options consist of correction of any abnormalities in the diet, injection of vitamin E and possibly provision of selenium. Administration of vitamin E is unlikely to result in toxicity and would be best before pathological change has occurred.

STOMATITIS

(Figs 7.47, 7.49–7.51, 11.80, 11.81)

Aetiology

Viral infections

Most cases of stomatitis are presumed to have an underlying viral aetiology, especially in situations where disease occurs as an outbreak, or where only particular species in a mixed-species collection are affected. Both iridovirus and herpesvirus have been implicated as possible causative agents. Extensive references to viral agents associated with stomatitis and other disease are given earlier in the Clinical Pathology chapter of this book. Reports of herpes-virus-associated stomatitis vastly outweigh reports of iridovirus-associated stomatitis.

Difficulties encountered when attempting virus isolation in cases of herpes-virus-associated stomatitis, including those in the pilot transmission study described by Origgi (2001), have led to the suggestion that the virus may only be present in oral secretions transiently, during primary infection or recrudescence. Some epithelial pathology may result from disruption of the

blood supply to oral epithelia (Origgi & Jacobson 2001: personal communication).

Herpesvirus infections are believed to lie dormant in CNS and other tissues following primary infection. Recrudescence in the post hibernation period is the most common time of presentation, because at this time many animals will be leucopaenic, energy deficient, inadequately heated and dehydrated.

Cunningham (2000) suggests that chelonian infection with iridovirus may follow exposure to amphibians carrying the agent. Chelonians are therefore at potentially greater risk if there is a pond or water source, inhabited by frogs or other amphibians, nearby.

Secondary infection of animals initially infected by virus is highly likely.

Non-viral infections

Infections accused by other agents in immunosuppressed animals are also possible (bacterial disease, mycotic infection). As the mouth is rich in opportunist bacteria and fungal agents it would be a logical place for an infection to arise in leucopaenic, malnourished and inadequately heated animals. Such a situation is common in poorly maintained animals following long periods of hibernation (Fig. 7.49). Almost all stomatitis cases exhibit abnormally high bacterial numbers on cytology.

Trauma and other causes

Hibernating recently fed animals may result in putrefaction of food remnants in and around the mouth. This may predispose to post-hibernation stomatitis.

Penetration injuries from food items may allow introduction of infections.

Local irritation—exposure gingivitis may cause local inflammation (drinking caustic fluid, eating chemically treated/affected food).

Clinical signs

Stomatitis is commonly referred to as stomatitis/rhinitis/conjunctivitis complex, as these clinical signs are common and appear in combination.

Herpesvirus and iridovirus infections in terrestrial tortoises are commonly associated with a variety of signs including:

- oedematous swelling of the ventral neck;
- yellow diphtheritic membrane formation (possibly on the mucosa of the tongue, oropharynx and nasopharynx);
- dysphagia;
- hypersalivation;
- occasionally animals are dyspnoeic;
- occasionally animals have a nasal discharge;
- conjunctivitis and ocular discharge are possible;
- exfoliation of the skin of the head and neck has occasionally been described;
- cases may also be septicaemic and leucopaenic following a prolonged period of hibernation.

Concurrent dehydration is common. Material from herpesvirus affected animals has shown that the central nervous system and organs such as the liver, kidneys and gonads may also be infected. Depression and neurological signs observed are therefore also likely to be virus related.

Not all animals in an outbreak of stomatitis/rhinitis/conjunctivitis complex will demonstrate clinical signs. The existence of clinically normal carrier animals is likely. This phenomenon appears to be species specific.

The incubation period of some diseases may be measured in years. Predisposing stressors may be necessary to cause clinical disease. Disease often appears to relate to recrudescence following immunocompromise, so that the 'incubation period' is variable or even indeterminate. Several cases presented to this author have been isolated for in excess of 25 years and present with no history of stomatitis or rhinitis. It is presumed that herpesvirus has been latent all this time.

Differential diagnoses include other infectious agents such as mycoplasmosis, immunosuppression, metabolic disease, hibernating recently fed animals, penetration injuries from food items, local irritation and chemical intoxications.

History

Chelonians may be predisposed to stomatitis when immunosuppressed for any reason.

Causes of immunosuppression include:

- inappropriate hibernation (inadequate conditions, preparation and duration);
- leucopaenia (e.g. follicular stasis);
- nutritional disease (e.g. hypovitaminosis A);
- metabolic disease (e.g. the uraemia/hyperuricaemia of renal failure);
- inadequate environmental provision (e.g. maintained in inappropriately cold and dark gardens without supplementary heat and light).

The history may give evidence of last exposure to animals potentially harbouring viral agents. Recent mixing with tortoises that had not been quarantined is reported to have occurred prior to several disease outbreaks. Disease often affects one specific species within mixed-species collections (Jacobson *et al.* 1985; Kabisch & Frost 1994; Marschang *et al.* 1997a).

Disease may be categorised into primary or recurrent infections (recrudescence) by the length of time animals have been isolated prior to onset of clinical signs. Primary infections are likely to occur within about eight months of mixing. Braune *et al.* (1989) found signs of stomatitis two to four weeks after naïve animals were exposed to new tortoises. Animals experiencing recrudescence have often been isolated for many years. This author has seen stomatitis in cases isolated for over 25 years.

Diagnosis

Stomatitis lesions are pathognomonic. Determining the aetiology is far more complex. This author (SM) regards all cases of stomatitis as having viral-associated disease until evidence is produced to the contrary.

Methods of diagnosing a viral infection and suitable sampling techniques are discussed under Clinical Evaluation and Clinical Pathology. Complementary information is also provided in this section.

Evidence of a viral infection may not be forthcoming unless the quality, timing and site of sampling are fortunate, and specific efforts are made to look for evidence of a viral aetiology.

Inadequate sampling technique may result in false negative results.

Even where a viral agent is identified in diseased individuals, this still doesn't mean it is the *cause* of any disease. Viral particles may appear as contaminants, incidental findings or they may be attracted to lesions from other sites. Transmission studies may be required to produce conclusive evidence of viral pathogenicity and these may not be appropriate to everyday situations in general practice.

Molecular tests and immunochemistry are likely to become available to United Kingdom clinicians in the near future. PCR for herpesvirus has recently become available by Laboklin Veterinary Laboratory in Bad Kissingen, Germany.

Evidence supporting a viral aetiology may be obtained from:

- history;
- clinical signs;
- cytology;
- immunohistochemistry;
- histopathology;
- electron microscopy (EM);
- virus isolation, serology, polymerase chain reaction (PCR examination of cell cultures, tissues, secretions and lavages);
- transmission studies.

Treatment

See also Viral disease in this section of the book for more specific advice on prevention and therapeutic options. It is unrealistic to eliminate many viral agents such as herpesvirus from infected chelonians. In such cases therapy should be centred upon encouraging the animals' own natural defences to deal with the agent, and upon eliminating stressors likely to predispose to recrudescence. Many animals will make a good recovery with appropriate nursing, especially if there is no other serious concurrent disease.

Reduce spread of infection

- Isolate affected animals.
- Barrier nurse animals, wearing disposable aprons and gloves, regarding them as highly infectious.
- Use appropriate disinfectants and disinfectable vivaria.

Optimise environment

- Basking lights, UVB fluorescent strip lights, heat provision and humidity should all be carefully controlled.
- Hospitalisation may be required for prolonged periods of several weeks.

Prevent and treat secondary infection

An antibiotic and/or antifungal agent is indicated.

Antiviral drugs

This author has observed subjective, but positive results when treating with acyclovir (Zovirax 200 mg/ml, Wellcome). Earlier texts advised a dose of 80 mg/kg, but this may be sub-therapeutic (McArthur 2000b). The author now employs a dose of 30–80 mg/kg, three times daily by stomach or oesophagostomy tube. Treatment is usually until remission of signs. Monitoring blood levels would be a method of ensuring therapeutic levels (contact local hospitals). No adverse reactions have been noted in approximately 100 animals treated.

Eliminate parasites

Debilitated specimens benefit from the elimination of parasites.

Analgesia and welfare

Analgesics should be considered, as this is a painful condition. An oesophagostomy tube will greatly assist patient management and decrease handling, especially where long-term fluid and nutritional support, or oral medication with drugs such as acyclovir, are required.

Correct underlying disease

As stomatitis may be due to recrudescence of carried disease, a full clinical examination, blood biochemistry and haematology assessment, and other investigations are all indicated. Concurrent disease should be treated as appropriate.

Fluids

Correct dehydration and provide maintenance fluids. Fluids at a rate of 0.5%–2% body weight/day in ml) and nutritional support, including the traditional diet as described earlier, should be provided. Animals should be bathed daily.

Nutritional support

Debilitated cases can be given liquidised diet or proprietary support diet (e.g. Critical Care Formula®, Vetark, UK, at the recommended dilution and a rate of 3 ml/100 g/day in divided doses). Nutritional support should be continued until cases are observed to be eating well. In most cases placement of an oesophagostomy tube will be beneficial.

Lesion care

The mouths of all clinical cases with evidence of stomatitis can be cleaned and debris removed once daily, using a cotton bud and povidone-iodine solution (USP) 7.5% w/v. This will require analgesia or anaesthesia. This solution may be cleaned off a few minutes after application. Such supportive care may be required for weeks or even months.

UPPER RESPIRATORY TRACT DISEASE (URTD)/RUNNY-NOSE SYNDROME (RNS)

(Figs. 7.48–7.51, 7.54, 7.60, 7.61)

Aetiology

This section is a clinical summary intended to assist the clinician when dealing with a case.

Virtually any species may be affected, but nasal discharge is common in most *Testudo* spp., and most free-ranging terrestrial tortoises from North America.

The aetiology of chelonian URTD has been open to a great deal of speculation over the past three decades with viral, bacterial and mycoplasma agents all coming under investigation. As our ability to screen populations for various types of agent improves, we are realising that mixed viral, mycoplasma and bacterial infections are relatively common in all species presented, and URTD is probably a disease of multiple aetiologies.

Clinical disease often occurs as outbreaks within colonies and is especially common in the post-hibernation period, when animals are immunocompromised, or following mixing of infected and immunologically naïve stock (e.g. as a result of introductions into a colony).

Causative agents may include:

- viral infections (e.g. herpesvirus);
- mycoplasmosis (e.g. *Mycoplasma agassizii*);
- mixed infections (especially *Mycoplasma agassizii* and herpesvirus);
- bacterial infections (no primary pathogenic bacterial agents have yet been identified as causative agents of URTD. However, secondary opportunist infections are common.);
- mycotic infections (as for bacterial infections);
- chlamydial infections, although the single published report in terrestrial chelonians centres more upon lower respiratory tract pathology;
- hypovitaminosis A, and inappropriate humidity and temperature provision, are implicated as possible causative factors.

Clinical signs

Nasal discharge is common to all cases of URTD. Serous, sanguineous and purulent discharges are all common at this author's surgery (SM). However, not all animals with a nasal discharge are necessarily suffering from upper respiratory tract disease. The anatomical connection between the mouth and upper respiratory tract through the choana means that any condition resulting in excessive salivation will result in saliva discharge through the nares. Chronic nasal saliva discharge is likely to act as an irritant, and inevitably produces a moist environment that promotes secondary infection. Animals that are poorly maintained and immunocompromised are therefore at great risk of secondary infections.

Nasal discharges can be caused by a number of aetiological factors, as we have seen. In some tortoises a specific cause may not be identified, despite extensive investigation. Nasal discharges can be unilateral or bilateral and this may correlate with the aetiology in some cases.

Signs associated with URTD include:

- abnormal posture;
- inactivity;
- anorexia;
- lethargy;
- discharge from the eyes;
- discharge from the mouth;
- discharge from the nares;
- lightening of tissues around the nares;
- increased or abnormal respiratory sounds;
- concurrent disease.

History

Disease often follows recent exposure to chelonians presumed to be carrying an infectious agent. Standards of husbandry are often poor.

Diagnosis

As URTD has various possible aetiologies, a variety of diagnostic techniques can be applied in order to determine a possible cause (Figs 6.26–6.28).

It is essential to review all aspects of husbandry and nutrition as well as to examine previous exposure to other chelonians potentially carrying infectious agents. The general health of the animals should be determined through blood assessment, diagnostic imaging techniques, and a comprehensive physical examination.

Where efforts to pursue a specific infectious agent are required, the following diagnostic investigations may be helpful:

- endoscopy (possibly retrograde through an oesophagostomy incision);
- biopsy (endoscopic or direct);
- cytology and histopathology;
- electron microscopy (nasal flushes and scrape biopsies);
- virus isolation (nasal flushes and scrape biopsies);
- serology (e.g. herpesvirus);
- *Mycoplasma* isolation (requires specific harvest techniques, transport media, storage and transportation of submission material);
- molecular tests (e.g. herpesvirus PCR and *Mycoplasma* PCR);
- microbiological culture (to determine secondary bacterial or fungal infections).

These are discussed in detail in the sections dealing with Clinical Evaluation, Clinical Pathology and Diagnostic Imaging, and in the Viral disease and Stomatitis sections in this chapter.

Treatment

Depends upon likely causative agent. Improvements in husbandry may help the animal's natural resistance to any agent present.

It is unrealistic to expect to eliminate agents such as herpesvirus from infected chelonians. In such cases therapy should be centred upon encouraging the animal's own natural defences to deal with the agent, and to eliminate stressors likely to predispose to recrudescence.

Where *Mycoplasma agassizii* has been isolated or identified by PCR, antimycoplasma therapy is indicated. Appropriate systemically administered drugs may include enrofloxacin, tylosin, doxycycline or clarithromycin. None has yet proved to be completely effective in the management of *Mycoplasma*-associated URTD. All are discussed in more detail in other sections of this book.

Mixed infections are common. This author has isolated *Mycoplasma agassizii* and herpesvirus from many United Kingdom animals affected with URTD (personal data on file). Agents have been found in both ocular and nasal swabs. These findings are consistent with the data collected by Mathes *et al.* (2001). In mixed infections treatment may be best geared towards improving the immunity of affected animals and reducing the possibility of disease spread. Animals should be hibernated with caution, and regular nebulisation may prove less stressful than frequent handling and direct local application of therapies. Further survey studies of United Kingdom colonies using PCR have also identified a significant number of positive normal animals. The suggestion is that carriage is common and cofactors may be necessary to produce disease.

Flushing the nasal chambers with medications able to control *Mycoplasma* infection (such as doxycycline, tetracyclines, enrofloxacin, clarithromycin and F10CL Standard Concentrate Disinfectant® (Interhatch, UK)) has been effective in dissipating signs associated with URTD. Subsequently the health and

demeanour of the patient has improved (Chitty 2002: personal observation). A technique for flushing the nasal chambers is illustrated in Fig. 7.61, nebulisation is illustrated in Fig. 9.18.

Summary

- appropriate antimicrobial, antimycoplasma or antiviral agents (local or systemic);
- improve/optimise standards of husbandry and nutrition;
- consider nebulisation with antibiotics or chemicals such as F10CL Standard Concentrate Disinfectant® (Interhatch, UK) or bioflavonoids (Propolis drops®, Bee Health, UK);
- supportive nursing;
- isolate and barrier nurse affected individuals.

VIRAL DISEASE

(Figs 7.45–7.72)

Aetiology

Chelonians appear to be highly susceptible to viral disease. Various reports describing pathogenic and non-pathogenic infections are given earlier in the Clinical Pathology section of this book. Herpesvirus is the virus most commonly reported, but there are also accounts of iridovirus, papilloma virus, and pox virus. Recent reports now also implicate further agents such as adenovirus, flavivirus and a *lytic agent* (Heldstab & Bestetti 1982; Jacobson *et al.* 1982a; Müller *et al.* 1988; Westhouse *et al.* 1996; Marschang *et al.* 1997a; Marschang *et al.* 1997b; Marschang *et al.* 1998a & b; Muro *et al.* 1998a; Orós *et al.* 1998; Drury *et al.* 1999a & b; Marschang *et al.* 1999; Origgi 1999; Une *et al.* 1999; McArthur 2000c).

Clinical signs

Variable. Occasionally only one species in a mixed-species collection is affected. The most common is stomatitis, but signs of viral disease also include:

Stomatitis

Stomatitis cases have been associated with viral particles using electron microscopy, virus isolation and serology (Harper *et al.* 1982, Jacobson *et al.* 1985, Cooper *et al.* 1988; Braune *et al.* 1989; Lange *et al.* 1989; Müller *et al.* 1990; Oettle *et al.* 1990; Kabisch & Frost 1994; Westhouse *et al.* 1996; Pettan-Brewer *et al.* 1996; Marschang *et al.* 1997a & b; Muro *et al.* 1998; Origgi 1999; Marschang 1999; Drury *et al.* 1999a & b).

Characteristic clinical signs:

- oedematous swelling of the ventral neck;
- yellow diphtheritic membrane formation on the mucosa of the tongue, oropharynx and nasopharynx;
- dysphagia;
- hypersalivation;
- occasionally animals are dyspnoeic;
- occasionally animals have a nasal discharge.

Skin lesions

Skin lesions with characteristic appearances are recorded in several viral disease outbreaks, especially within marine species. However, similar signs may also occur with non-viral disease.

Signs of viral disease include:

- Grey-patch disease in *Chelonia mydas* is associated with characteristic papules and spreading grey patches seven to eight weeks after hatching (Rebell *et al.* 1975).
- Fibropapillomas in *Chelonia mydas* with green turtle fibropapillomatosis (FP) are considered pathognomonic of the disease. However, it is not yet clear if this is a viral disease.
- Papillomas are described in *Platemys platycephala* by Jacobson *et al.* (1982a). Papilloma-like viral particles were demonstrated by electron microscopy.

Signs not specific to viral disease in terrestrial species include:

- Exfoliation of the skin of the head and neck is described in herpes-virus-related necrotic stomatitis (Braune *et al.* 1989).
- Oettle *et al.* (1990) describe eczema-like skin lesions in the hindquarters of three *Chersina angulata* tortoises during an outbreak of disease presumed to be of viral origin. However, it is possible that these lesions were iatrogenic.
- Orós *et al.* (1998) found pox-virus-like particles in periocular, papular skin lesions in a captive *Testudo hermanni*.

Ocular changes

Ocular changes are not specific to viral disease. However, characteristic ocular lesions have been noted in some disease situations where a viral aetiology was strongly suspected.

Reports of ocular signs:

- Jacobson *et al.* (1986) describe disease associated with herpesvirus-like particles where 14–24-month-old *Chelonia mydas* presented with caseous exudate over the eyes. The disease was termed lung, eye, trachea disease (LETD) because of clinical signs.
- In green turtle fibropapillomatosis (FP), corneal and periocular fibropapillomas are described by Jacobson *et al.* (1989) and Herbst *et al.* (1994). Periocular fibropapillomas may interfere with eyesight, disrupt feeding and other behaviour and increase the risk of predation.
- An ocular discharge was described by Jacobson *et al.* (1985) during a herpesvirus infection of *Geochelone chilensis*.
- Oettle *et al.* (1990) record 'occasional panophthalmitis' during an outbreak of suspected viral disease.
- Westhouse *et al.* (1996) reported a mucoid oculonasal discharge during iridovirus-associated URTD in *Gopherus polyphemus*.
- Blepharitis and superficial keratitis were reported, by Muro *et al.* (1998a), in three *Testudo graeca* during an outbreak of herpes-virus-related disease that also resulted in stomatitis and chronic rhinitis.
- Corneal opacity and prolapse of the *membrana nictitans* was noted in the acute stage of a herpes-virus-associated disease, with lymphoid tissue proliferation in *Testudo hermanni* (Drury *et al.* 1998). Whitening of the cornea was noticed during the acute phase of the infection. The whitening cleared over the following week.
- Yellow papular lesions of the eyelids were noted in a *Testudo hermanni* that died in captivity in Spain (Orós *et al.* 1998). Pox virus was demonstrated using electron microscopy.

Respiratory disease

Dyspnoea and rhinitis are not specific to viral infections. However, many reports of both upper and lower respiratory tract diseases in chelonians are associated with the presence of viral particles.

Upper respiratory tract disease was linked to a herpesvirus infection in *Testudo graeca* by Muro *et al.* (1998). At our clinic, episodes of upper respiratory tract disease are common in colonies of *Testudo* and other species that have experienced outbreaks of stomatitis and fatalities in preceding years.

Various reports of lower respiratory tract diseases associated with the presence of virus-like particles have been described (Cox *et al.* 1980; Jacobson *et al.* 1986; Müller *et al.* 1988; Müller *et al.* 1990; Pettan-Brewer *et al.* 1996; Westhouse *et al.* 1996; Drury *et al.* 1998; Marschang *et al.* 1998b).

Cases of unexplained respiratory disease should be regarded as having a possible viral aetiology, especially where they occur as an outbreak.

Neurological signs

Neurological signs are not specific to viral disease. Hind limb paresis has been reported in *Psammobates tentoria, Homopus areolatus* and *Chersina angulata*, during an outbreak of disease of probable viral origin (Oettle *et al.* 1990). Drury *et al.* (1998), McArthur (1998) and McArthur (2001c) describe transient hind and forelimb paresis in *Testudo hermanni* and *Geochelone pardalis* affected by a herpes-virus-like infection associated with disseminated visceral lymphoproliferation.

Anorexia

Anorexia is not specific to viral disease although many authors describe anorexia related to it (Frye *et al.* 1977; Heldstab & Bestetti 1982; Jacobson *et al.* 1982b; Müller *et al.* 1988; Braune *et al.* 1989; Oettle *et al.* 1990; Kabisch & Frost 1994; Westhouse *et al.* 1996; Pettan-Brewer *et al.* 1996; Marschang *et al.* 1997b).

Other signs

The following signs are not specific to viral disease:

- hepatitis (Jacobson *et al.* 1982a; Heldstab & Bestetti 1982);
- nephritis (Müller *et al.* 1990);
- generalised debility (various);
- acute haemolytic episodes and lymphoproliferative disease (McArthur 2001c).

Sudden death

Sudden death is, of course, not specific to viral disease. Viral disease may, though, present in a wild population as an increased death rate.

Cooper *et al.* (1991) point out that sudden deaths/fatal epidemics in the absence of evidence of viral infection should not be considered definitively viral. This publication was in response to cases documented by Highfield (1990) where deaths and clinical signs consistent with viral disease in United Kingdom captive chelonians were extensively recorded.

History

Any of the following may apply:

- stressors (wild capture, excessive stocking rates, parasitism, starvation, inappropriate husbandry);
- immunocompromise (poor temperature provision, nutritional deficiencies, starvation, poorly-managed hibernation);
- mixing of populations with differing degrees of immunity;
- exposure to amphibians (especially with respect to iridovirus);
- exposure to blood-sucking vectors such as ticks.

Diagnosis

Histopathology

Intracytoplasmic or intranuclear inclusion bodies strongly suggest the presence of a viral infection. These may be noted in impression smears, biopsy samples, scrapes and necropsy samples. Histopathology samples may be more reliable than cytology in revealing the presence of inclusions as preparation involves sectioning of nuclear material, whereas cytology samples spread cytoplasm about an intact and membrane-covered nucleus. Occasionally, inclusions are present in cells for metabolic or degenerative reasons and some inclusions are parasitic.

The stain affinity and location of inclusion bodies may suggest that a viral agent is present:

- basophilic cytoplasmic inclusions (haematoxylin and eosin) are compatible with iridovirus infection;
- eosinophilic intranuclear inclusions (haematoxylin and eosin) are compatible with herpesvirus infection;
- immunohistochemistry stains may soon improve the sensitivity and specificity of both cytology and histopathology in the detection of specific viral antigens (Origgi 1999).

Specific histopathological changes such as diphtheritic membrane formation are strongly suggestive of viral disease.

The nature of cellular infiltrate may suggest a viral aetiology.

Ballooning degeneration of cells and syncytium formation may occur in the presence of viral disease.

Cytology and immunohistochemistry

Immunocytology, based upon the molecular detection of antigens using immunoperoxidases, may be a useful tool in the diagnosis of herpes-virus-related stomatitis (Origgi 1999).

Cytology and/or histopathology may also detect any bacterial or mycotic agents present.

Serology

Serology for chelonian viruses may involve virus neutralisation (VN) or ELISA for the presence of herpes-virus antibodies (Origgi 1999).

Sampling technique

As methods of serological diagnosis are likely to advance rapidly over the next few years, it would be wise to contact laboratories offering serological testing for chelonian herpesvirus regarding their current submission requirements. At the time of writing, this author uses heparinised jugular blood samples, which are then centrifuged and the plasma subsequently removed by pipette. Following decanting the plasma is frozen and stored or transported chilled to the laboratory for testing (virus neutralisation).

Molecular tests

Most swabs kept under refrigeration, frozen material or formalin-preserved histopathology submissions are ideally suited to viral PCR screening. As PCR is likely to be highly sensitive to the presence of viral DNA, it should result in fewer false negative results than electron microscopy, serology or virus-isolation techniques. However viruses may only be found at specific times during the

course of infection and may not be shed in normal carriers. These complications limit the interpretation of negative PCR test results.

Faecal, oral and serum PCR tests are now available in both Europe and the United States (Marschang 1999: personal communication; Origgi 2000: personal communication).

Evidence of retrovirus may result from the use of a retroviral DNA probe (Venugopal 1998: personal communication).

The presence of characteristic herpes-virus base sequences was first demonstrated by Une *et al.* (1999) using PCR during examination of tissues from stomatitis lesions during an outbreak in *Malacochersus tornieri* and *Testudo horsfieldi.*

Electron microscopy (EM)

Visualisation of virus-like particles within biopsy, scrape, smear, urine, faeces, discharge or centrifuged homogenate is strongly suggestive of viral disease. However, failure to find viral particles does not rule out the presence of a viral infection. Even after a virus has been isolated within a cell line and is producing an extensive cytopathic effect, it may be impossible to visualise using EM if it is only present in very small numbers. This may be the case with the lytic agent.

Sampling

Material for examination by electron microscopy can be obtained by direct collection of excreted body fluids, such as faeces and urine, or by washes to release such material (e.g. respiratory or cloacal washes). Biopsy and necropsy samples are well suited to electron microscopy and can be obtained at post mortem, endoscopic examination or surgically. Fluid from vesicles can be harvested using aspiration through a sterile syringe and needle.

During the course of a viral infection, electron microscopy can be used to identify the presence of viral particles, but only if sufficient numbers of viral particles are present and the sample material is harvested from specific locations at specific times. Serial sampling during the course of the disease increases the likelihood of obtaining a positive sample. After collection, samples should be chilled or frozen during storage and transport. Fixatives are not usually required, although sample material should not be allowed to desiccate. Care should be taken to avoid unnecessary contamination of samples by contact with human or other tissues.

Virus isolation

Isolation of virus from diseased chelonians can allow viral infections to be diagnosed in situations where particles are too scarce to be revealed by EM. If cytology or histopathology gives results consistent with a viral infection, then submission of swabs for virus isolation may be justified, even in the absence of positive EM evidence.

Sampling

Samples for virus isolation can be harvested from dry swab specimens of lesions or lumenal surfaces, tissue biopsies or tissues collected at biopsy. An aseptic sampling technique is always advisable. Endoscopic biopsy to collect visceral material may be helpful. Care should be taken not to allow different tissues to contact one another, and to prevent contamination with human viruses (from clinicians).

Once harvested, material should be placed in sterile sealed containers or bags, and transported to the virology laboratory chilled. Further advice should be sought from the virology laboratory. If samples are not kept chilled then the virus is lost and a false negative result may be obtained. Freezing may also reduce the ability to isolate viral DNA, but may not affect PCR results.

Many workers have now isolated viral agents from diseased chelonians (Biermann & Blahak 1994; Biermann 1995; Kabisch & Frost 1994; Marschang *et al.* 1997a; Marschang *et al.* 1998a & b; Drury *et al.* 1999a & b; Marschang 1999; Origgi 1999). As chelonian virology techniques improve, and species-specific cell lines become more available, a better understanding of the culture requirements of specific viral agents is emerging.

Treatment

It is unrealistic to expect to eliminate many viral agents such as herpesvirus from infected chelonians. In such cases therapy should be centred upon encouraging the animal's own natural defences to deal with the agent, and eliminating stressors likely to predispose to recrudescence.

To prevent spread of infection, maintain in small closed groups (<10) and practice disciplined quarantine and screening procedures.

Barrier nurse all sick chelonians, as though they are capable of shedding agents highly infectious to others, especially those with clinical signs as described above (McArthur 2000b).

Nursing

Environmental support

Optimise husbandry during recovery. The provision of a suitable therapeutic environment is essential when nursing any debilitated chelonian.

Temperature, humidity, light and ventilation should all be suitably controlled. Material and equipment should not be shared between tanks unless adequately disinfected. In situations where both aquatic and semi-aquatic chelonians are hospitalised, care should be taken to ensure that drainage and discharge of tank water cannot disseminate disease.

This author hospitalises sick chelonians in isolation, in easily disinfected plastic or glass tanks. Such tanks are cheap and effective. All vivaria are provided with maximum–minimum thermometers and humidity gauges, UVB tubes, basking lamps and background heat sources as appropriate.

Semi-aquatic and marine chelonians generally benefit from hospitalisation in isolation within suitable tanks. Aquatic chelonians can also be individually nursed in treatment tanks if they are available. In an emergency, a bath or shower unit may prove temporarily suitable.

Fluid therapy and systemic support

In acute viral disease administration of intraosseous and/or epicoelomic fluids may be convenient and effective. Oral fluids can be given temporarily by stomach tube, but the placement of a semi-permanent oesophagostomy tube will avoid excessive handling and reduce the transfer of infectious agents from the oral cavity into the digestive tract.

Improving patient nutrition is beneficial in situations where husbandry has been poor or animals have been anorexic.

Medications

At our clinic, individuals displaying overt signs consistent with viral disease, such as necrotic stomatitis, are generally treated with a combination of medications.

Evidence of the presence of herpesvirus (PCR, serology, isolation, histopathology) encourages the use of acyclovir. This author (SM) is comfortable with its use in cases of stomatitis. Where cytology demonstrates the presence of large numbers of yeasts and bacteria in combination with inflammatory cells, the clinician should consider the use of antimicrobials.

Antivirals

Subjectively, this author has had positive results treating terrestrial tortoises suffering from stomatitis with acyclovir (Zovirax®, Wellcome) at a dose of 80 mg/kg three times daily by stomach or oesophagostomy tube (240 mg/kg/day). The author has also used acyclovir in upper respiratory tract disease in *Testudo graeca* and systemic disease in *Geochelone pardalis*, again with positive results.

Initial work by the author comparing acyclovir at 80 mg/kg SID with water, in a group of 24 animals with stomatitis/rhinitis/conjunctivitis complex, where later herpesvirus and mycoplasma were both isolated, did not indicate any improvement in the medicated group above the control (McArthur 2000b). This author now prefers to use acyclovir at a minimum dose of 200 mg/kg in divided doses when treating herpes-virus-related disease, but conclusive evidence of efficacy is not yet available.

The duration of acyclovir treatment used by the author (SM) depends upon the condition of the patient and the rate of recovery. Often cases of stomatitis are treated for three to six weeks by oesophagostomy tube. This author frequently doses *Testudo* spp. for periods of up to three months, without obvious adverse reaction. Species dosed for many weeks throughout their recovery with no apparent complications related to medication include *Geochelone elegans, Testudo horsfieldi, Testudo graeca, Testudo marginata, Testudo graeca, Testudo ibera* and *Geochelone pardalis*.

Treatment with acyclovir appears to be most effective when commenced early in the course of suspected viral disease. We have seen no adverse reactions to acyclovir to date.

Acyclovir may be more effective when used to treat primary infection as opposed to recrudescence. No pharmacokinetic data is available to support adequate gut absorption in chelonians following oral dosing. Sub-therapeutic dosing may encourage viral resistance. Acyclovir used in over 100 sick animals has not impeded treatment and has not been associated with any noticeable unfavourable reactions. No data is yet available to conclusively demonstrate acyclovir therapy is efficacious in the face of chelonian herpes-virus infection.

There is a theoretical risk of acyclovir resistance (and related resistance to antiviral agents with a mode of action also dependent upon viral thymidine kinase) following excessive prophylactic or sub-therapeutic dosing. Gut absorption of acyclovir in man is limited, at 15%–30%. Absorption is presumed to be capacity limited, suggesting that frequent dosing may be more effective than a single bolus dose. Adverse reactions associated with oral therapy in man are restricted to less than 5% of patients. Such reactions included nausea, vomiting, diarrhoea, rash, stomach pain and headache, but were similar in placebo-treated patients.

It may be better to give antiviral agents to chelonian herpesvirus carriers prior to hibernation. The likelihood of molecular screening tests becoming available in the near future means that potential cases of recrudescence might become identifiable allowing prophylactic antiviral therapy with agents such as interferons. The use of interferons and bioflavonoids in the prehibernation period may be less likely to induce viral resistance than the use of acyclovir. Further research into their potential in the treatment of chelonian herpes-virus infection is warranted (McArthur 2000b).

Antibiotics

If there is evidence suggesting the presence of a bacterial infection, an antibiotic such as enrofloxacin (Baytril®, Bayer) is often given at 5–10 mg/kg IM/SC/PO. Dosing frequency varies with species. *Geochelone* spp., such as leopard tortoises, are reported by some clinicians to become distressed, salivate and become hyperactive following injection with enrofloxacin. These animals appear to tolerate epicoelomic injection of enrofloxacin diluted 1:10 with saline without such complications.

Alternatively, other agents such as ceftazidime (Fortum®, Glaxo) may be given at 20 mg/kg every 3 days IM.

Prophylactic antibiosis is justified in cases where septicaemia and bacterial infections are likely.

Any leucopaenic animal may benefit from broad-spectrum antibiosis.

Antifungals

If there is evidence of a fungal infection, an antifungal agent such as ketoconazole (Nizoral, Janssen) may be given at 15–30 mg/kg SID orally. Antifungal agents should be considered as a complement to long-term antibiotic therapy. Some agents are potentially hepatotoxic. Doses for antifungals are given in the Therapeutics section.

Antiparasitics

Debilitated specimens benefit from the elimination of parasite burdens. Often chronically ill tortoises suffering from viral disease presented to this author have severe burdens of both helminths and trichomonads.

Lesion management

In terrestrial chelonians with stomatitis this may involve mouth care, oesophagostomy tube placement and removal of necrotic material and oronasal discharges. General anaesthesia and/or analgesia may be indicated.

Euthanasia

There are situations where euthanasia of chelonians suffering from viral disease should be performed in order to prevent suffering or to reduce the spread of disease. A protocol for the euthanasia of a terrestrial chelonian is given elsewhere in this book (see Chapter 14).

Prevention

Stocking density

Commercial transportation and high stocking densities may expose large numbers of individuals to virus during importation and trade, particularly if latent carriers relapse into disease and/or

shed virus. Wherever tortoise colonies are grouped together in double figures, this author advises subdividing them into smaller units of no more than ten.

Parasitism
Excessive levels of parasitism may predispose to outbreaks of disease. This should be avoided through screening and treatment.

Nutrition
Inadequate or unsuitable nutrition may result in lowered disease resistance and encourage recrudescence.

Environment
An unsuitable environment may result in unnecessary stress. Care should be taken to correct inadequate temperature provision, inadequate photoperiod, inadequate light intensity/spectrum and inadequate humidity or ventilation.

Inappropriate hibernation (duration, preparation, technique and conditions) may result in immunocompromise predisposing to recrudescence of carried disease.

Water quality
Diseases of aquatic and semi-chelonians may be exacerbated by the stress of unsuitable water temperatures, overstocking and poor water quality.

Exposure to infection
Diseased individuals should be removed from chelonian collections as quickly as possible and nursed in isolation. They should never be introduced into a healthy colony. Serology and certain molecular tests may prove an effective method of assessing which chelonians are best isolated from others.

Recovered and recovering chelonians may be returned to contact with others only when they are perceived not to put the remaining animals at risk. This requires evidence of immunity to the agent concerned. Jacobson *et al.* (1999a) gives guidelines to the management of captured diseased chelonians.

Disease is most likely when animals harbouring viral agents are mixed with immunologically naïve individuals with differing background and different origins. Avoid unnecessary mixing of different tortoise species. Disease severity is often increased when a species barrier is crossed.

Vectors and fomites
Tagging devices, microchip-implanting devices, enclosures and feeding equipment should be sterilised or disinfected wherever possible before contact with new animals. Ectoparasites should be eliminated.

Excessive handling of eggs or repeated use of the same substrate in incubators may increase the exposure of hatchlings to disease.

When nursing sick chelonians, barrier nursing, disposable gloves and individual disinfectable vivaria should be employed wherever possible.

Quarantine
Before introducing new chelonians into a stable colony, it may be wise to mix them with a small group of sentinels first, to see if any disease becomes apparent in either sentinels or newcomers over the next 12–18 months. If both old and new stocks remain disease free then exposure of new individuals to a larger group may be cautiously increased.

The author currently suggests that keepers isolate any new chelonians before introducing them into an existing colony. A quarantine period of 12–18 months is suggested. Alternatively, keepers must accept any disease outbreaks that result. However, it must be remembered that it may be several years before latency and carriage are revealed and quarantine cannot be regarded as foolproof.

History of incoming stock
If additions are to be made to a stable colony, or a healthy tortoise is being sought a mate, it is wise to make enquiries regarding the general health of any source colony before accepting new stock from it. Good tortoise breeders will generally provide the following information when asked:

- What deaths and what diseased animals required treatment over the preceding 12–18 months?
- What interactions have new stock experienced with other chelonians over the previous12–18 months?
- In cases where the stock is not being purchased from the breeder, it is wise to discover how long the source has known the stock. For some reptile dealers this may be a week or less.

Screening of incoming stock
Soon it may be possible to screen tortoises for prior exposure to herpesvirus using PCR and serology tests. It would be unwise to mix seropositive/PCR positive and seronegative/PCR negative stock. All seropositive chelonians are best regarded as carriers of disease. False positive and negative tests could result in disaster!

Colony management
It is wise to subdivide captive tortoises and turtles into small isolated groups. This will limit the spread of infectious disease. This author currently advises that colonies should be prevented from reaching double figures wherever possible. In most species, numbers of less than five would be ideal.

It is wise to try to maintain chelonians in individual species groups and to resist the temptation to mix species and populations together.

Isolation
Ensure tortoises are kept isolated from others if taken to a site where other tortoises are gathered. Examples include pre-hibernation and post-hibernation examinations.

Ill tortoises should be removed from a colony as quickly as possible. This is very important if clinical signs are consistent with infectious disease (e.g. breathing difficulties, unexplained jaundice, unexplained lethargy and inactivity, anorexia, increased oral and nasal secretions or even sudden death).

It is unwise to rescue and nurse sick chelonians in the presence of other healthy chelonians without practising a very high standard of hygiene and disinfection.

Barrier nursing
Sick chelonians should be nursed away from healthy specimens, and if possible at another site, with a high level of hygiene and disinfection.

Vaccination

- The isolation of chelonian herpesvirus and iridovirus gives the possibility of future vaccination against chelonian viral diseases agents. Recently attempted vaccination of a group of 50 *Testudo* sp. with an inactivated non-adjuvanted vaccine was not especially encouraging (Marschang *et al.* 2001).

WEIGHT ABNORMALITIES—OVERWEIGHT

Aetiology

- Oedema: heart disease, renal disease, excess fluid therapy, hypoalbuminaemia (hepatic/renal/anorexic).
- Overfeeding/inactivity.
- Coelomic masses (follicular stasis, cystic calculi, gravidity, egg retention, tumours, abscesses).
- Coelomic effusion (hepatitis/coelomitis/bladder rupture).

Clinical signs

- Clinical signs are generally non-specific e.g. bloating, inactivity, swelling/distension of the prefemoral and/or cervical fossae. Other signs will depend upon the aetiology.

History

A complete history is required. Charts of expected weights and lengths may be helpful, but are not entirely reliable as a guide to health. A chart of weight against time is helpful.

Diagnosis

Diagnosis relies upon further investigation to determine the cause. A complete physical examination and work-up are indicated.

Treatment

Treatment depends upon the aetiology.

WEIGHT ABNORMALITY—UNDERWEIGHT

Aetiology

Underweight is due to a negative energy balance or excessive loss of fluids (dehydration). Juvenile animals that are fed protein-rich diets and maintained in warm conditions, will occasionally experience unsuitably fast growth rates in consequence, and will be inadequately calcified. Common causes of underweight are:

- starvation;
- disease-related anorexia;
- inappropriate environmental;
- inappropriate diet;
- inter- or intra-species aggression and behavioural anorexia.

Clinical signs

Correct body weight for age and size in captivity remains a subjective judgement. Breed charts of expected weights and lengths may be helpful, but are only a guide. None have yet been validated in peer-reviewed literature.

History

A complete history is required. It is often non-specific and may relate to inappropriate diet or environmental provision. A chart of weight against time, if available, is helpful.

Diagnosis

Calculations proposed by some to determine ideal body weight were discussed previously, under Clinical Evaluation. Diagnosis relies upon further investigation to determine the cause.

A complete physical examination and work-up are indicated.

Treatment

Treatment depends upon the aetiology:

- Underlying disease should be diagnosed and treated appropriately.
- Intensive fluid therapy and nutritional support using products such as Critical Care Formula® (Vetark, UK) and/or a liquidised diet typical of the species may be required for relatively long periods of nursing.
- Where the animal is distressed by handling or recalcitrant, oesophagostomy tube placement should be considered.
- Environmental provision should be optimised, inappropriate temperature, humidity, light and social exposure should be corrected.
- The previous diet should be checked for suitability.

YOLK COELOMITIS

Aetiology

Coelomic trauma can result in follicular rupture and produce severe yolk coelomitis (Rosskopf & Woerpel 1982; Frye 1991). Other factors, such as follicular stasis and infections, may also precipitate follicular or ovum rupture, and result in release of yolk material into the coelomic cavity.

Yolk, albumen and embryonic tissues are irritant and induce a considerable inflammatory reaction. Spillage results in a florid, fibrinous yolk serosocoelomitis characterised histopathologically by lipid-laden histiocytic macrophages, numerous heterophils and lymphocytes (Frye 1991).

Frye describes this as a post-ovulatory condition due to trauma to ovulated eggs, but it is also possible that damage, degeneration, inflammation and infection of preovulatory follicles result in yolk coelomitis (Fig. 11.105).

Needle aspiration of coelomic contents of mature female chelonians should be approached with caution, especially in the peri-hibernation and immediate post-hibernation periods when follicular activity in captive chelonians is strong. Puncture of ovarian follicles may result in yolk coelomitis.

Diagnosis

Rosskopf & Woerpel (1982) suggest that yolk coelomitis should always be considered in the differential diagnosis of post-partum illnesses of chelonians. They report that such cases are characterised by a heterophilia and raised levels of AST, plasma proteins, and LDH. Confirmation is suggested using coelomic endoscopy (laparoscopy).

According to Frye (1991), yolk coelomitis can be diagnosed by a history of straining, a decline in condition and radiographic evidence of the presence of one or more fractured eggs, or their fragments, within the coelomic cavity.

Yolk coelomitis may also occur as a complication of coelomic surgery e.g. during ovariosalpingectomy or salpingotomy for treatment of follicular stasis or egg retention.

Cytology of the coelomic fluid of mature females with unexplained anorexia may reveal a diagnosis of yolk coelomitis. However care must be taken, as trauma to follicles during sampling must be avoided. Similarly, laparoscopy may also allow visualisation of the coelomic contents and follicles for evidence of rupture or disease.

Treatment

Rosskopf & Woerpel (1982), Frye (1991) and Raiti (1995) all give a poor prognosis when yolk coelomitis is present. Often such tortoises are already critically ill before presentation.

Raiti (1995) advises treatment by coeliotomy, ovariectomy, and coelomic lavage using a dilute povidone-iodine solution (Betadine diluted 1:10 with water). Saline is also an appropriate fluid for coelomic flushing. Coeliotomy, retrieval and removal of egg material, and coelomic lavage are suggested by (Frye 1991), who generally gives a guarded prognosis in such cases.

Culture and sensitivity of coelomic contents are indicated if cytological analysis indicates the presence of a possible infection. Suitable antimicrobial therapy can then be given.

14 ANAESTHESIA, ANALGESIA AND EUTHANASIA

STUART MCARTHUR

ANAESTHESIA

Indications for anaesthesia in chelonians include:

- immobilisation for diagnostic procedures;
- immobilisation for therapeutic/surgical procedures;
- analgesia for surgical procedures and wound/trauma management.

Development of agents suitable for reptilian anaesthesia continues. Many of the drugs and doses recorded later are included to help establish a historical database from which informed choices can be made. Inclusion of an agent does not mean it is advised or suggested as being appropriate. Some agents have only been used in limited species, and many anaesthetic possibilities remain unexplored (Figs 14.1–14.24).

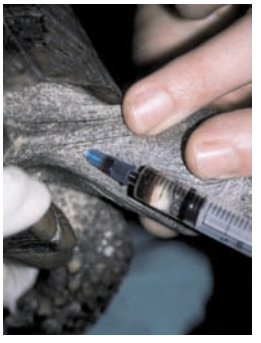

Fig. 14.1 Intravenous injection of alphaxalone/alphadolone into the jugular vein (*Testudo graeca*).

Fig. 14.3 Injection of propofol (Rapinovet®, Schering-Plough; Diprivan®, Zeneca) into the dorsal venous sinus of a male marginated tortoise (*Testudo marginata*). This site requires considerable disinfection and, whilst often convenient, may not be as suitable as the jugular vein (see section on Venepuncture).

Fig. 14.2 Injection of low dose ketamine into the dorsal venous sinus of a female painted turtle to facilitate examination of the head and neck of an aggressive and reclusive animal. Here access to cranial intravenous sites is impractical.

Fig. 14.4 Intravenous injection of propofol (Rapinovet®, Schering-Plough; Diprivan®, Zeneca) into the sub vertebral sinus of a juvenile spur-thighed tortoise (*Testudo graeca*).

(a) (b)

Fig. 14.5 Alphaxalone/alphadolone (Saffan®, Schering-Plough Animal Health): induction after IV injection is rapid and may take as little as 30 seconds. Anaesthesia is generally smooth and uneventful. Recovery at 10–20 minutes post IV injection tends to be reliable. Repeat doses for maintenance result in predictable extension of anaesthesia.

Fig. 14.6 Endotracheal intubation: a shortened urethral catheter, giving set tubing, catheter for intravenous injection or an uncuffed endotracheal tube can be used to intubate the trachea.

Occasionally intubation is assisted by pulling the tongue forward with protected forceps and inserting a tube through the glottis, found centrally $^2/_3$ of the way down the tongue. The tongue does not come out of the mouth.

Localisation of the glottis can be facilitated by pushing with the finger tip into the intermandibular space in a craniodorsal direction.

The glottis may be anaesthetised using a cotton-tipped applicator soaked in a local anaesthetic.

Fig. 14.7 Zinc oxide tape can be used to secure the endotracheal tube to the lower jaw, or around the head as shown.

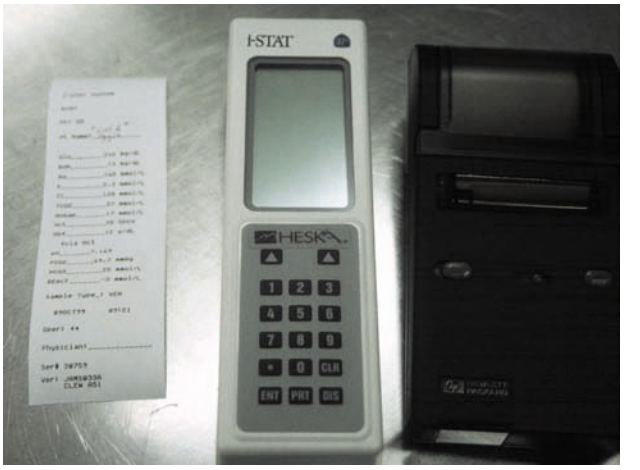

Fig. 14.8 Blood gas measurements are available in general practice. The I-Stat® (Heska, Fort Collins) is capable of measuring pH, pCO_2, pO_2, sO_2, TCO_2, HCO_3 and base excess, at the animal's side.

Fig. 14.9 Huntleigh Mini Dopplex® (Huntleigh, Cardiff) with an 8 MHz pencil probe.
(J. Chitty 2002)

Fig. 14.10 Huntleigh Mini Dopplex® (Huntleigh, Cardiff) with an 8 MHz flat probe.
(J. Chitty 2002)

GENERAL CONSIDERATIONS

Hypothermia

Hypothermia is a form of immobilisation devoid of analgesia. It compromises the gastrointestinal microflora and decreases metabolism and the immune response, possibly even resulting in enteritis and septicaemia (Johnson 1991). In older texts, hypothermia is inappropriately described as a method of anaesthesia. Induced hypothermia should never be employed. Freezing will be painful.

Fig. 14.11 The Doppler pencil probe is ideal for access to the heart, as it can be passed under the shell very easily. The small footplate allows auscultation of different parts of the heart. In particular, the atrioventricular valves can be picked out and murmurs detected. However, this probe cannot be left in place so the anaesthetist must periodically reposition or alternatively hold it.
(J. Chitty 2002)

Fig. 14.12 The Doppler flat probe is not ideal for cardiac assessment as it is too large. However, it is well suited to anaesthetic monitoring, as it can be positioned over a vessel such as the carotid artery and taped in place.
(J. Chitty 2002)

Pain and analgesia

Bennett (1998b) reviews pain and analgesia in reptiles and amphibians and points out that reptiles possess appropriate anatomy and physiological structures for nociception. Our ability to interpret pain in reptiles is limited and our knowledge of how to relieve it is equally poor. Mader (1998c) points out that we do not understand why reptiles fail to remove themselves from heat sources that are burning their tissues. It may be that the reptile is feeling pain but is unaware that moving away from a heat source will cause it to decrease. Pain may manifest itself as inactivity, as a failure to feed or behave normally, or it may be expressed in altered behaviour following what an observer perceives to be a painful stimulus.

It is every veterinarian's responsibility to eliminate pain from animals under their care. It is unacceptable to restrain reptiles physically without analgesia in order to undertake painful or stressful procedures.

Fig. 14.13 Pulse oximetry provides a pulse rate and an estimation of peripheral arterial oxygen haemoglobin saturation (Vet-ox 4404®, Heska, Fort Collins, USA).

Pulse oximetry technique and hardware have not yet been validated in chelonians. It should be used to complement other methods of patient monitoring.

Fig. 14.14 Skin sensors can detect blood flow and haemoglobin colour through the skin of some chelonians but are less reliable than oesophageal or cloacal probes.

Anatomy and physiology

The chelonian glottis is positioned caudally in the tissue of the tongue. It remains closed during states of rest and a laryngeal dilator muscle opens the glottis to allow breathing (Johnson 1991). Intubation through the glottis of small terrestrial tortoises is relatively easy in both conscious and anaesthetised animals. In larger tortoises and marine turtles, where a rapid recovery may be desirable, authors such as Moon & Stabenau (1996) advocate induction of anaesthesia by inhalation of volatile agents following direct, conscious intubation, using gags. According to Bennett (2000) this is fairly difficult, as large marine turtles may only breathe every 10–20 minutes and significant force may be required to pass an endotracheal tube after gag application.

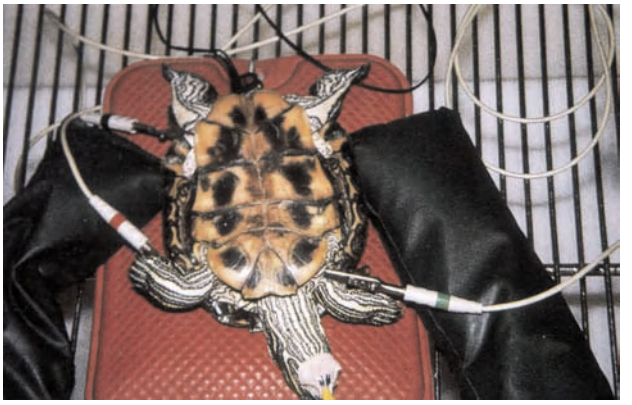

Fig. 14.15 ECG monitoring gives evidence of patient heart rate but is of little use in the assessment of anaesthetic depth or patient stability. Little information is available on chelonian ECG interpretation although its use is advised in early texts.

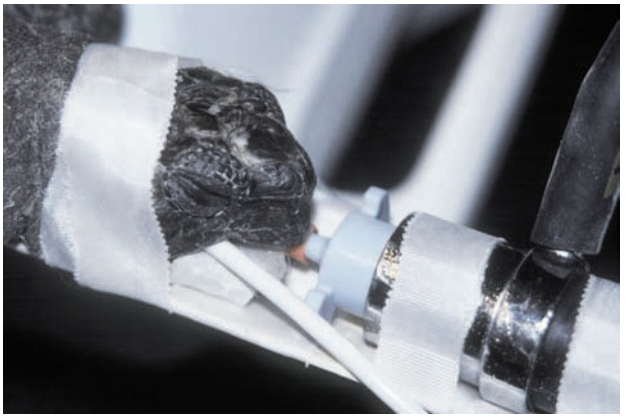

Fig. 14.16 Connections of equipment to the head are easily stabilised by splinting the head, monitoring or ventilating hardware and anaesthetic circuit to a tongue depressor with zinc oxide tape. This prevents everything from moving apart during surgery and ensures seals are kept airtight.

Fig. 14.17 Automatic ventilation (Mark 3 Small Animal Ventilator®, Vetronic Services, UK).

Chelonians have a short trachea with complete cartilage rings. Division into paired bronchi is in the cranial 1/3 of the cervical area. It is easy to intubate most chelonians unilaterally by mistake. Unilateral intubation should be avoided if possible, although this author is not aware of any reported undesirable consequences in anaesthetised chelonians.

Fig. 14.18 Automatic ventilation (Mark 3 Small Animal Ventilator®, Vetronic Services, UK).

Fig. 14.19 In recovery, the patient is returned to ventral recumbency as soon as possible post operatively in order to maximise ventilation of lung tissue. Tidal volume is compromised in dorsal recumbency.

Ventilation can be continued automatically until the patient is breathing on its own.

Reptiles generate negative pressure in the lungs by two methods: increasing the size of the coelomic cavity; contraction of smooth muscle within the lungs. In chelonians, movement of the pelvic and axillary limbs and muscles alters the volume of the coelomic cavity. This is central to effective ventilation. These movements are decreased during anaesthesia and intermittent positive pressure ventilation (IPPV) should be administered during all chelonian anaesthetic procedures, either manually or by a ventilator. Placing a chelonian in dorsal recumbency will further compromise ventilatory movements because of visceral weight reducing the lung volume, unaided tidal volume, and blood perfusion.

Bennett (1998a) suggests that chelonians can perceive a low oxygen environment, become apnoeic, and convert to anaerobic

Fig. 14.20 Warmth and maintaining core body temperature at or above 26°C are crucial to a speedy anaesthetic recovery. Here heat is available from the basking lamp, plastic bottles and a microwave heat pad. Limbs remain protected from inadvertent trauma.

Fig. 14.21 Inspiration: extension of the neck and/or limbs increases available respiratory volume.

Fig. 14.22 Expiration: limb and/or neck flexion will increase expiration by further decreasing available respiratory volume.

metabolism. Such physiological mechanisms as anaerobic respiration and cardiac shunting may be useful in diving and hibernation, but complicate inhalation anaesthesia and euthanasia techniques reliant upon a prolonged period of anoxia.

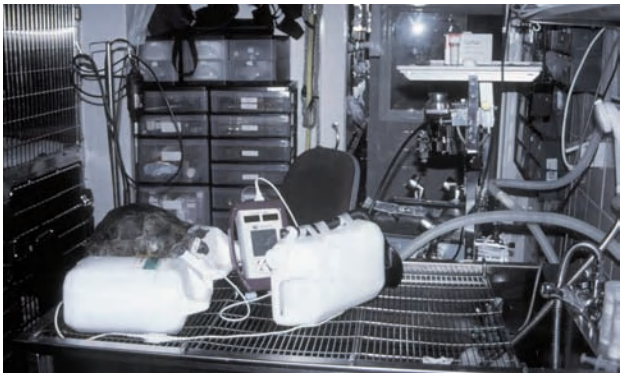

Fig. 14.23 The anaesthetic recovery area of the author's clinic (SM).

Fig. 14.24 Animals sedated for examination or procedures should be kept warm during recovery. Here sedated animals are temporarily taped to hot water bottles until they are making voluntary movements.

Crossley *et al.* (1998) examined anaesthetised turtles (*Pseudemys scripta*) and found that hypoxia elicited an increase in resistance of pulmonary blood vessels. Decreased elimination of inhalation agents primarily excreted by the lungs, such as isoflurane, could therefore be expected in diving turtles where a physiologically induced increase in pulmonary arterial resistance may reduce elimination of volatile agents through the lungs.

It is not clear how physiological signals trigger alterations in pulmonary vessel size in reptiles. Diethelm & Mader (1999) found that recovery times in anaesthetised iguanas, induced and maintained using isoflurane, were shorter when air (as opposed to oxygen) was administered using IPPV throughout the recovery. It would appear likely that circulatory shunting prevented pneumonic excretion of isoflurane in animals ventilated with oxygen, suggesting that high oxygen tensions also altered pulmonary circulation in these animals. Further work is necessary to clarify the mechanisms affecting excretion of volatile anaesthetics such as isoflurane in reptiles.

STAGING ANAESTHESIA

Brogard (1987), Wood *et al.* (1983) and Malley (1999) describe assessment of the stage of anaesthesia in chelonians (Table 14.1).

PATIENT ASSESSMENT

All patients should undergo a pre-anaesthetic examination. A more extensive guide to the assessment of health in chelonians is given in the chapter on Diagnosis.

Table 14.1 Stages of anaesthesia (Wood *et al.* 1983; Brogard 1987; Bennett 1998a; Malley 1999).

Stage 1	Slow movements, odd placements of the limbs, a positive righting reflex, response to painful stimuli, muscles are not relaxed.
Stage 2	Few spontaneous movements, poor righting reflex, response to painful stimuli reduced, light muscle relaxation.
Stage 3	No movements, no righting reflex, marked to complete muscle relaxation, no voluntary muscular response to surgical procedures.
Stage 4	*This represents medullary depression approaching death.* Loss of the corneal blink reflex (touch or blow on the cornea) and loss of vent reflex indicate excessive anaesthetic depth. Care should be taken to avoid corneal trauma during reflex assessment. A significant decrease in heart rate may indicate excessive anaesthetic depth.

General health

An examination should be performed in order to determine the patient's health. Wherever possible, diseased patients should be stabilised to minimise any anaesthetic or surgical risk.

A pre-anaesthetic blood assessment is helpful. Bennett (1999) suggests that haematological assessment, blood biochemistry assessment, faecal examination, radiographic examination and microbiological evaluations should all be performed before attempting anaesthesia, unless the anaesthesia is required as a life saving measure. These procedures are described in more detail elsewhere in this book and form the ideal pre-anaesthetic work-up.

Hydration status and recent fluid management

The patient's hydration status should be assessed prior to considering anaesthesia. Unmanaged dehydration is a contraindication to anaesthesia. Dehydrated patients should be stabilised by administration of fluids. This can be through intravenous, intracoelomic and intraosseous or oral routes. Fluids are best given prior to anaesthesia or other stressful procedures.

Assessment of dehydration in chelonians is somewhat subjective at present. Malley (1997) suggests that clinical signs associated with dehydration in reptiles include reduced skin turgor, increased skin folding, sunken eyes, reduced body weight and alterations in blood biochemistry and haematology values. Ion pumps and significant fluid reabsorption in the lower urinary tract and digestive tract complicate the use of many clinicopathological parameters as indicators of hydration status. Various parameters such as PCV, urea, albumin, uric acid, and potassium may reflect changes in hydration status (see hydration Clinical Pathology section).

The frequency with which a chelonian urinates may decrease with dehydration or inadequate fluid provision. It is good to have access to data recording the frequency of urination over the previous 10 days or so. A patient that has not urinated despite 7–10 days' active fluid therapy should be anaesthetised with great caution if at all.

Where the hydration status of a patient is unknown, this author would advise hospitalisation within a managed vivarium environment during any pre-anaesthetic stabilisation. At the author's clinic, 5–20 ml water/kg would be administered daily in divided amounts, in combination with soaks, to such patients. Fluids would normally be given by stomach tube, although any of the routes mentioned earlier may be employed, depending on the clinical situation.

Where clinical signs such as sunken eyes or biochemical parameters such as PCV suggest significant dehydration, the oral, epicoelomic (pectoral/sub-plastral), intraosseous and intravenous routes may all be employed and 0.5%–4% of body weight may be given. A common maximum fluid administration figure suggested in reptilian literature appears to be 4% of body weight over 24 hours. In our experience it is seldom necessary to administer this much to a chelonian. We would work on around 1%–2% being given in the first 24 hours and 0.5%–1% per 24 hours for maintenance thereafter.

Fluid therapy before during and after anaesthesia helps to maintain hepatic function and renal excretion rates. It maximises the metabolism and excretion of anaesthetic agents by the kidneys, and may promote a rapid, uneventful recovery from anaesthesia. (Assessment of hydration status is also described in Clinical Evaluation and Clinical Pathology.)

Observation of the unstressed patient

Demeanour, activity, frequency of urination and defecation and appetite may give some idea of patient stability and health. It may be appropriate to hospitalise a chelonian for a short period in order to establish behaviour in a vivarium environment accurately with suitable fluid provision, humidity, lighting and nutrition.

Resting respiration in particular should be evaluated and this should include the number of breaths per minute, head and neck posture and depth of respiration. Observations may reveal signs suggestive of respiratory disease.

Body weight

Medications such as injectable anaesthetics are often drawn up according to body weight and then given to effect. Adjustment should be made for patients that are considered underweight or overweight in any medication protocol. Sedgwick (1988) points out that the percentage of fat, and percentage of shell to lean weight in significantly underweight animals, should be reflected in dosages of injectable agents for sedation or anaesthesia, although an initial calculation of dosage based upon total body weight including shell is a suitable starting point.

Various equations have been derived in an attempt to assess body condition in chelonians (Jackson 1980b; Jacobson *et al.* 1999a; Donoghue 1996; Mader 1998a). Calculations can be used to assess pre-anaesthetic patient condition in addition to the other points raised here, but often they are applicable to only one species. This author (SM) has doubts as to the benefits of using such equations in any species.

Species differences

Differences have been noted in the susceptibility of different

species to anaesthetic agents. Anaesthesia in musk turtles (*Kinosternon* spp.) has been induced using inhalation agents in gaseous induction boxes but was not induced in other semi-aquatic chelonians, such as the red-eared slider (*Trachemys scripta elegans*).

Giant tortoises may require a lower dose of injectable agent per unit body weight than smaller ones (Crane *et al.* 1980).

The injectable benzodiazepine midazolam sedates some species, but not others (see later).

Many species appear capable of intrapulmonary blood shunting (shunting blood away from the lungs whilst submerged). This may decrease both absorption and excretion of volatile anaesthetic agents.

Where differences in response to specific agents are known they are described later.

PATIENT PREPARATION

Bennett (1998a) suggests that reptiles should be acclimatised to new environments such as veterinary hospitals, and should be maintained at an optimal temperature and humidity for several days before anaesthesia. Often the observations of body functions by keepers are inadequate for the assessment of general health. In such cases this author advises a stabilisation period of up to 5 days for observation and assessment before anaesthesia is attempted—unless immediate intervention is crucial to saving the patients life. Some authors disagree and advocate rapid assessment and surgery, particularly where hospital facilities are rudimentary.

Pre-anaesthetic fasting is not advised, as aspiration with a closed or intubated glottis is unlikely and it takes several days to decrease the intestinal volume of hind gut fermenters (Bennett 1995).

Care should be taken to avoid exposing chelonians to excessive desiccation from operating theatre lights. Covering the patient with appropriate drapes and moistening of aquatic and semi-aquatic species is advised.

Temperature

It is advisable to anaesthetise reptiles at temperatures within their appropriate temperature range (ATR) and preferably at or near their preferred body temperature (PBT) (see earlier notes on thermal terminology, p. 93) where normal metabolic and behavioural activity are optimum (Cunningham & Gill 1992).

Reptiles should not be confined in a manner rendering them unable to thermoregulate. Where suitable vivaria are unavailable, care should be taken to maintain body temperature using a heat source such as a basking lamp, heat pad or hot water bottle. Where feasible, the anaesthetic induction room, operating room and recovery room should all be within the ATR of the species concerned. Most well-maintained chelonians achieve their preferred body temperature through external behavioural means. At our surgery it is common practice to stabilise the patient within its ATR and around its PBT for several days before contemplating general anaesthesia. This stabilisation forms part of an initial health plan following admission.

According to Sedgwick (1988), Pokras *et al.* (1992), Bennett (1998a) and Malley (1999), heart rate can be used as an indictor of thermal acclimatisation. An equation to determine a heart rate (HR) indicative of stabilisation at a suitable temperature indicative of PBT is given by Pokras *et al.* (1992), although it is not clear that this equation can be applied to every reptile species.

$$\text{HR of reptile} = 34\ (\text{wt in kg})^{-0.25}$$

Bailey & Pablo (1998) point out that insufficient data is available to validate the equation and the derivation of the equation is not explained. They suggest it is inadequate for predicting heart rate in reptiles. Sedgwick (1988) gives a table of heart rate in comparison to body weight. Both the equation and the table suggest that PBT is a subjective judgement, because neither considers species, health or seasonal influences.

Sedgwick (1988) suggests that a suitably-stabilised small reptile weighing 0.05–5.0 kg should achieve a pre-anaesthetic cloacal temperature of 20°C or above, and large reptiles of 5.0–100 kg should achieve a pre-anaesthetic cloacal temperature of 18°C or above.

If a clinician is uncertain of patients' ATR and PBT, exposure to an environmental temperature range of 24°C–28°C during pre-anaesthetic stabilisation seems to work well for most species encountered in general veterinary practice. An ambient temperature below the ATR will affect the metabolism and/or excretion of anaesthetic agents and other drugs and this may result in a prolonged recovery from anaesthetic. Hypothermic patients are also more likely to experience unstable anaesthesia and present difficulties in effective monitoring.

A recovery temperature in the upper region of the ATR or above may increase tissue oxygen demands above rate of supply and therefore destabilise the patient.

Some temperatures suggested by different workers in the field are:

- Schildger *et al.* (1993) suggest preheating reptiles to temperatures between 25°C–30°C prior to anaesthesia.
- Sedgwick (1988) suggests 30°C–35°C for 'reptiles', however this may be a little warm for some chelonians.
- Page (1993) suggests 26°C–32°C to be a suitable range for maintaining anaesthetised reptiles.
- This author recommends an ambient room/patient anaesthesia and recovery temperature of 24°C–28°C during the anaesthesia of most chelonians and advocates the use of a cloacal temperature probe to monitor patient core temperature during anaesthetic procedures.

Fluid therapy

Fluid therapy in chelonians is inadequately described in the literature, and it is hard to provide definitive rules regarding its use during anaesthetic procedures. Fluids should be given routinely to any dehydrated chelonian, especially in the stabilisation period prior to general anaesthesia. Johnson (1991) suggests intravenous fluid administration during anaesthesia at a dose of 5 ml/kg/hour and Bennett (1998a) 5–10 ml/kg/hour.

Bennett (1998a) suggests that hypotonic fluids may be beneficial in reptiles but comments that isotonic solutions also appear clinically effective. It would seem that hypotonic fluids may be good for rehydration in some reptiles, but could result in tissue oedema if given to well-hydrated chelonians during general anaesthesia. Our clinic favours the use of isotonic epicoelomic fluids for maintenance, as described in the Therapeutics section.

Local anaesthesia

Chelonian skin is sensitive to traumatic pain, and local anaesthetics have proved suitable when inducing analgesia during minor procedures (Cooper 1976). Lignocaine HCL 1%–2% (Lidocaine), with or without adrenaline (Johnson 1991), or procaine at 1% (Burke 1986), can be infiltrated locally (e.g. with a 25 G–27 G needle). Care should be taken to avoid excessive injection of these agents as they may affect local blood supply and interfere with wound healing at sites where surgery is performed.

There is potential for local anaesthetics to interfere with cardiac activity. Using a mammalian equivalent, as little as 0.25 ml of a 2% lignocaine (Lidocaine) solution would reach the mammalian toxic dose in a 500 g tortoise. The toxicity of this agent in chelonians is unknown, but personal experience has shown that animals are severely depressed when, for example, lignocaine is absorbed through the oral mucosa when applied to the glottis prior to intubation. Regional nerve blocks may be of use.

INDUCTION

Anaesthesia has been successfully induced and maintained using both injectable and volatile agents. Anaesthesia can be maintained by administering incremental doses of the induction agent or by intubating the patient and ventilating with a volatile agent. Techniques for the induction of inhalation anaesthesia include anaesthetic chambers/open drop boxes, facemasks and forced endotracheal intubation (Bonath 1979; Bennett 1998b).

Gaseous induction using anaesthetic chambers/open drop methods or masking down is inappropriate for most chelonians. They may breath-hold for long periods or switch to anaerobic respiration, and they can utilise cardiac and intrapulmonary shunting. *Trachemys scripta elegans* has been shown to survive up to 27 hours in a 100% nitrogen environment (Johlin & Moreland 1933). Bennett (1998b) suggests that chelonians can perceive a low oxygen environment, become apnoeic and convert to anaerobic metabolism.

One study exposed box turtles (*Terrapene carolina* and *Terrapene ornata*), red-eared sliders (*Trachemys scripta elegans*) and snapping turtles (*Chelydra* spp.) to high concentrations of halothane for 60–90 minutes. Anaesthesia was not produced, presumably because of prolonged breath holding (Brannian *et al.* 1987). Similar exposure of mud turtles (*Kinosternon* spp.) resulted in anaesthetic induction because these turtles fail to breath hold.

In some situations, such as an intervention in a wild marine turtle necessitating a rapid recovery in order to return it to water, forced intubation and volatile induction may be justified (Moon & Stabenau 1996). It remains unknown if injectable induction agents might reduce cardiovascular or intrapulmonary shunting away in comparison with forced intubation. Forced intubation and induction using volatile agents was considered successful in the terrestrial *Gopherus agassizii* by Rooney *et al.* (1999).

When using an anaesthetic agent for the first time in a reptile, a veterinarian is forced to rely upon a combination of the literature and anecdotal evidence of suitability from colleagues. Familiarity with agents over a long period allows relatively accurate prediction of induction, maintenance, and recovery times.

PATIENT MONITORING

It is commonly said that there is no such thing as a safe anaesthetic, only safe anaesthetists. Safe anaesthetists cannot be replaced with monitoring devices. Monitoring devices merely complement the anaesthetic recording and monitoring of the anaesthetist. Occasionally monitoring devices give misleading readings.

Ventilation

It is not possible to monitor the depth of anaesthesia in chelonians using ventilation movements, as the shell prevents costal ventilation and ventilation through movements of the limbs is absent during anaesthesia. The rate at which the animal is manually ventilated should be monitored. Ideally it should attempt to match the rate in the conscious patient (see also Ventilation below).

Reflexes

Withdrawal, palpebral, corneal and 'pain' reflexes are all of some use in assessing degree of anaesthesia. During surgical anaesthesia the only external sign of life may be the corneal blink reflex and the vent reflex (movement of the tail or leg in response to squeezing the vent).

Righting

Loss of righting reflex is of use in the assessment of many anaesthetised reptilians, however it is hard to assess in most chelonians due to their flattened shape. They may be unwilling to move as a result of fear and other factors. Malley (1999) suggests the use of a pinprick to stimulate chelonians, but this may alter the validity of reflex assessment.

Withdrawal

Tail and limb withdrawal reflexes to pinprick or pinch are lost during surgical anaesthesia (Bennett 1998a).

Eye reflexes

Loss of palpebral reflex generally occurs at a light plane of anaesthesia (Bennett 1998a). According to Malley (1999) it is unreliable in the assessment of anaesthetic depth. Pupillary dilation occurs during surgical anaesthesia of many chelonians. Loss of the corneal blink reflex (touch or blow on the cornea) indicates excessive anaesthetic depth (Bennett 1998a).

Jaw tone and head withdrawal

Malley (1999) suggests jaw tone is reduced at stage 2 and absent at stage 3 of anaesthesia. Loss of head withdrawal reflex indicates arrival at a surgical plane of anaesthesia (Bennett 1998a).

Vent

Loss of vent reflex indicates excessive anaesthetic depth (Bennett 1998a).

Heart rate

According to Bennett (1998a), a decrease in heart rate may indicate excessive anaesthetic depth. However, a large number of other factors such as temperature will also influence heart rate, as described earlier, making this an unreliable parameter.

Equipment

Pulse oximeter

A pulse oximeter is a two-wavelength spectrophotometer that determines the colour of pulsating blood and equates this colour with oxygen saturation. It is suggested that the absorption spectrum of haemoglobin in the visible spectrum (400–700 nm) is similar among all vertebrates (Bailey & Pablo 1998). A lingual clip on the tongue, an oesophageal or a cloacal probe may all suit reptiles (Malley 1999; Mader 1999).

Allen (1999) points out that a pulse oximeter is best used over time to assess trends. Individual oxygen saturation values are of limited use in predicting actual blood oxygen levels. Some limitations based upon weak signals, poor placement, interference of the signal, slow signal averaging rates and optical shunting have been recognised (Saint-John 1992; Bailey & Pablo 1998). Bailey & Pablo (1998) point out that as little as 4%–8.6% of normal blood flow has been shown to allow a pulse oximeter to function, and that a strong output signal should not be interpreted as adequate blood flow or oxygen delivery.

This author uses a pulse oximeter during all chelonian anaesthetic procedures (Vet-ox 4404®, Heska). Pulse oximetry can be used in reptiles to provide a measurement of peripheral pulse rate and an estimation of arterial oxygen haemoglobin saturation (McArthur 1996b; Bennett 1998a; Bailey & Pablo 1998; Malley 1999), however pulse oximetry has not yet been adequately validated in chelonians.

In chelonians that had pre-ventilation anaesthetic core body temperatures above 25°C, using alphaxalone/alphadolone induction and ventilated with 2% isoflurane at four breaths per minute, pre-ventilation oxygen saturation values fall to as low as 58% and rise to as high as 95% post ventilation. It appears relatively easy to ensure that oxygen saturation is maintained above 65% when ventilating at this rate.

Perhaps surprisingly, most anaesthetised chelonians encountered by this author appear clinically unaffected by low SpO2 (blood oxygenation) and it is not yet clear if monitoring and ultimately controlling SpO2 increases the safety of chelonian anaesthesia.

Ultrasonic Doppler

An ultrasonic Doppler flow detector probe with an audible transducer can be placed over the carotid artery of small chelonians to give a measure of peripheral blood flow and heart rate (Sedgwick 1988). In larger chelonians, such as marine turtles, Moon & Stabenau (1996) suggest the crystal can be placed over the femoral vessels on the proximal medial aspect of the hind limb. For more detailed information see page 388.

ECG

The linear, three-chambered reptilian heart makes interpretation of an ECG complex (Holz & Holz 1994). An ECG trace can be obtained by the attachment of the leads to chelonian limbs (McArthur 1996b; Bennett 1998a; Malley 1999).

The anaesthetic depth at which an ECG change can be observed is likely to be too deep to make it useful in the assessment of anaesthetic depth (Bailey & Pablo 1998). When this is combined with the fact that the heart continues to beat for some considerable time after it has been removed from the body, it would appear that ECG monitoring is of very limited value. Heart rate may be more useful in assessing potential progressive anaesthetic overdose, and this is described later.

Oesophageal stethoscopes

Oesophageal stethoscopes provide a crude method of determining patient cardiac function. They are best used to monitor gradual changes over a period and the effect of anaesthetic duration on cardiovascular physiology. They are unreliable in the assessment of anaesthetic depth. Bailey & Pablo (1998) point out that oesophageal stethoscopes should be placed in intubated reptiles only, in order to prevent airway obstruction.

Blood gases

Blood gas measurements are available in general practice. The i-Stat® (Heska, Fort Collins, Colorado) is capable of measuring pH, pCO2, pO2, sO2, tCO2, HCO3, and base excess, at the animal's side, within two minutes. Ideally, arterial blood should be measured, but often this is impractical. Few superficial arteries (the carotid arteries and femoral arteries) are readily available without significant trauma to the patient. Useful results may be available from venous blood, but require cautious interpretation. The differences in oxygen tension in arterial and venous blood do not yet appear to have been documented. Temperature corrections may be required e.g. from 98°F machine calibration to a reptile's actual body temperature.

Cardiovascular system

The chelonian heart can be observed directly during a plastral coeliotomy in most cases. Direct visual assessment of the heart, mucous membrane appearance and blood appearance at points of surgically induced haemorrhage may provide evidence supportive of adequate cardiac output. Such assessment should correlate with pulse oximetry and ultrasonic Doppler assessments.

Blood loss

The purpose of monitoring blood loss is to anticipate intervention. Any blood loss in a small exotic species is significant, with the blood volume measuring only 7%–10% of the body weight.

Temperature

Elevating patient temperature to the upper region of the ATR during recovery may increase oxygen consumption. Therefore ventilation during recovery will minimise the risk of hypoxia (Bailey & Pablo 1998). Temperatures below the ATR will hinder drug metabolism and may prolong anaesthesia.

Patient temperature can be monitored using rectal or oesophageal temperature probes (Bailey & Pablo 1998). This author uses the probe attachment of the Vet-ox® 4404 (Heska).

Blood glucose

Bailey & Pablo (1998) suggest that small exotic species have limited glycogen stores. During prolonged anaesthesia and chronic disease these benefit from monitoring of blood glucose levels with appropriate supplementation where hypoglycaemic. It is

presently unclear if similar hypoglycaemic problems occur in small chelonians, but it is suggested that it does occur in debilitated, free-ranging marine chelonians.

Other parameters

End tidal CO_2 levels and blood pressure monitoring may soon prove useful and are presently under evaluation for use in reptiles.

8MHz DOPPLER

The Doppler device is designed for use in human medicine to assess blood flow into extremities. The 8MHz probe detects flow, in small to medium-sized vessels, up to about 2 cm below the skin surface. Lower frequency probes are also available for pregnancy diagnosis/egg assessment and for assessing deeper vessels. There are two probes currently used in veterinary medicine:

- *Pencil probe.* This is very small area probe, very good for detecting small vessels and for use in body fossae. It detects vessels at an angle of 45°; i.e. the vessel detected is not directly beneath the probe.
- *Flat probe.* This probe was developed for use in blood pressure measurement in other species. The larger area of the probe means it can only be used where there is easy access. However, it does detect vessels directly beneath the probe.

In general, the probe is attached to a loudspeaker or headphones so an audible signal is being received. However, it can also be used so a paper trace is produced. While this provides a permanent record, the trace is very hard to interpret.

The uses of the 8MHz Doppler are outlined below.

Anaesthetic monitoring

The probe detects blood flow. It can be used to detect flow in the heart, neck vessels or limb vessels. This allows heart/pulse rate to be detected audibly. Changes in rate and rhythm are easily heard. In addition, the intensity of the signal allows assessment of the 'strength' of heartbeat, i.e. the cardiac output.

When used on vessels in extremities, Doppler allows assessment of blood flow to these extremities and therefore tissue perfusion (especially when allied with pulse oximetry). If the anaesthetised animal becomes shocked or hypovolaemic, the strength of signal in the limbs will drop. Either probe may be used, however the pencil probe must be held in position, while the flat probe can be taped over a convenient vessel.

Diagnostic auscultation

The pencil probe allows the heart blood flow to be auscultated. Harsh murmurs produced by endocarditic lesions on the atrioventricular valves are easily heard in septicaemic reptiles. This finding is an important prognostic indicator.

Venepuncture sites

Arteries and veins often run together, and detection of an artery can be useful, as a suitable vein may be close. Veins may also be directly auscultated; the blood flow in these is much slower with less definite pulses, so the signal is much quieter and may be masked by an underlying artery. However, the two types of vessel are easy to differentiate. Tracing vessels with the pencil probe may also be useful when planning surgery: e.g. when placing an oesophagostomy tube (O-tube) the jugular veins and carotid artery may be traced and avoided. Use of the pencil probe is essential here as the area of the flat probe is too large to allow accurate tracing.

INTUBATION

Intubation and ventilation are advisable during all chelonian anaesthetic procedures. After appropriate induction, items such as a shortened urethral catheter, plastic giving-set tubing, plastic intravenous cannula, or an uncuffed endotracheal tube can be used to intubate the trachea.

Intubation is easily achieved by carefully pulling the tongue forward, e.g. with protected forceps. The tongue of a chelonian can't be pulled beyond the mouth and upward pressure of a digit below the jaw allows better visualisation. This allows the clinician to insert an endotracheal tube through the glottis, found centrally $^2/_3$ of the way down the tongue. Local anaesthetic (lignocaine HCL 1%–2%) can be applied to the glottis with a cotton swab to assist in intubation. This is seldom required unless forced conscious intubation is attempted. This author (SM) has encountered severe muscular, respiratory and cardiac depression when application of local anaesthetic agents to oral mucosa has been excessive (Figs 14.6–14.7).

It is usually possible to use ordinary connectors (e.g. Portex®, UK) to link endotracheal tubes to a circuit such as an Ayres T-piece in order to apply intermittent positive pressure ventilation (IPPV). Stabenau (1993) suggests that marine turtles can be intubated by passing a piece of strong plastic PVC tubing through the beak and into the glottis. This achieves a primary intubation allowing an endotracheal tube to be passed through its centre. This double intubation renders a turtle unlikely to damage the endotracheal tube with its keratinised jaws.

In order to decrease anaesthetic recovery times in wild marine chelonians requiring replacement into water, Moon & Stabenau (1996) investigated induction of anaesthesia by inhalation of the volatile agent isoflurane, following direct conscious intubation. Intubation was achieved using gags. A rapid recovery was not produced. One possible explanation was the redirection of blood away from the respiratory tract, decreasing the elimination of volatile agents through the lungs. Moon & Stabenau propose that the delayed recovery was a response to anoxia, but it would also seem plausible that the release of neuroendocrine substances during a stressful induction may increase the resistance of pulmonary vasculature to blood flow in diving species. Forced intubation has produced rapid induction and recovery times in *Gopherus agassizii* using sevoflurane. In this terrestrial species anaesthetic recovery was not prolonged—a finding consistent with the above hypothesis (Rooney *et al.* 1999).

VENTILATION

During anaesthesia, respiratory movements may be too weak to inhale and expel air. Assistance is therefore required to achieve

adequate ventilation. This author advises that intermittent positive pressure ventilation (IPPV) should be administered during all chelonian anaesthetic procedures.

As most chelonians anaesthetised by this author are manually ventilated throughout the whole of their anaesthetic period using a non-rebreathing circuit (e.g. Ayres T-piece), anaesthetic gas flow rate is of less importance than ventilation rate.

An appropriate rate to ventilate most anaesthetised chelonians does not yet appear to have been determined, but it would appear that 2–6 breaths per minute, or the normal resting respiratory rate of the species if known, can be used during the manual or automatic ventilation of most if not all chelonians. This author routinely ventilates small terrestrial chelonians at four breaths per minute.

Where injectable agents are used for the induction and maintenance of small terrestrial chelonians, intubation and manual ventilation with oxygen or air can be performed at four breaths per minute. Reptilian lungs are delicate and care should be taken when IPPV is used to avoid damage to the patient through over-inflation. This author currently employs automatic ventilation (Mark 3 Small Animal Ventilator, Vetronic Services, UK), although ventilation by hand or by the use of a human facial resuscitation (Ambu) bag are both effective too. Automatic ventilation produces a constant tidal volume, pressure and frequency and therefore results in increased anaesthetic stability.

Variable, excessive and inadequate ventilation rates may have detrimental effects on inhalation anaesthesia and prolong its duration. Ventilation/perfusion (V/Q) mismatch was suggested as an explanation for prolonged recovery times in Kemp's Ridley turtles (*Lepidochelys kempii*) induced and maintained using isoflurane inhalation (Moon & Stabenau 1996). The ability of marine turtles to shunt blood away from the lungs (intrapulmonary shunting) and avoid both absorption and excretion of anaesthetic gases must be considered during inhalation anaesthesia in diving chelonians. These authors ventilated sea turtles at 2–8 breaths per minute and proposed that the cardiopulmonary effects of surgical positioning, anaesthesia and mechanical ventilation were responsible for long recovery times. Atropine was not influential in altering the recovery time and the authors hypothesise that its administration had limited effect on cardiac shunting. Attempts to increase the CO_2 content of ventilated inspiratory gas appeared to result in a severe respiratory acidosis and so this practice had to be discontinued.

Further research measuring induction/recovery times and blood gas measurements are necessary to derive a ventilation rate most appropriate to different chelonian species with each anaesthetic regime. Such research might also throw further light upon the clinical significance of the reptilian dive reflex in various species investigated. The natural resting respiratory rate for the species would seem a wise starting rate for anaesthetic ventilation rate.

The nature of the carrier gas used to ventilate the anaesthetised chelonian may also have influence upon recovery rates with volatile anaesthetic agents. Comparisons of recovery rates when anaesthetised chelonians are ventilated with air as opposed to oxygen are not yet available, but anecdotal unpublished data suggests that recovery may be facilitated when ventilation is performed using air (Bennett 2000: personal communication; Divers 2000: personal communication). Decreasing oxygen levels increases respiratory frequency and increasing CO_2 levels increases tidal volume. Both factors work together to increase respiratory drive.

INJECTABLE ANAESTHETIC AGENTS

Injectable anaesthetic agents may be given by a variety of routes, require little equipment and are familiar to most veterinarians. It is unwise to generalise about their use as they differ greatly in their pharmacodynamics. In chelonians, propofol (Rapinovet®, Schering-Plough) and alphaxalone/alphadolone (Saffan®, Schering-Plough Animal Health) are best administered intravenously. Some agents may be effective by intramuscular (e.g. alphaxalone/alphadolone), intracoelomic or intraosseous (e.g. propofol) (Fonda 1999) routes.

Intravenous routes in chelonians include the right or left jugular veins, the subcarapacial sinus, the brachial venous plexus and the dorsal coccygeal vein or sinus (Richter *et al.* 1977; Samour 1984). There is some evidence that the anatomy of the chelonian dorsal coccygeal area is variable and occasionally injection in this area results in intravertebral sinus injection (Ippen & Zwart 1995). Jugular injection may be preferable.

Renal and hepatic portal systems are present in chelonians and are described elsewhere in this book. The specific effects of portal systems upon comparative excretion rates of anaesthetic agents injected into either the cranial or caudal portions of the body do not appear to have been investigated. It may have significance where the venous system of the tail is employed.

Atropine

Atropine sulphate has been used as a premedicant at 0.01–0.04 mg/kg IM in reptiles (Frye 1991a). However, the value of pre-anaesthetic medications in the prevention of anaesthetic induced bradycardia is unclear (Bennett 1998a). Attempts to reduce cardiovascular shunting using atropine in diving chelonians have not been successful (Moon & Stabenau 1996). Adverse effects, such as increased myocardial oxygen demand, are possible with this agent.

Phenothiazines

Acepromazine maleate (ACP)

Acepromazine maleate has been used in reptiles at a dose rate of 0.1–0.5 mg/kg IM (Frye 1991a). George (1997) and Whittaker & Krum (1999) used a mixture of acepromazine and ketamine intravenously to facilitate intubation and inhalation maintenance of sea turtles (10 ml ketamine 10% to 1 ml acepromazine 1%: ketamine HCL 100 mg/ml and acepromazine maleate 10 mg/ml). According to these authors, 8–12 mg/kg of the ketamine in this mixture was an effective dose in most species, but leatherback turtles (*Dermochelys coriacea*) required up to 15% more.

Chlorpromazine

Young & Kaplan (1960) found that 10 mg/kg chlorpromazine reduced the induction time of anaesthesia using pentobarbitone in *Pseudemys scripta*.

Diazepines

Midazolam

Several workers have investigated the use of the benzodiazepine midazolam (Hypnovel®, Roche) for the chemical restraint of chelonians (Bienzle *et al.* 1992; Holz & Holz 1994; Oppenheim & Moon 1995; Harvey-Clarke 1993). The use of this agent as part of a combination anaesthetic is described below in the section describing dissociative anaesthetics. Several authors suggest that midazolam has poor sedative qualities when given on its own and can be variable in different individuals.

There appear to be differences in the degree of sedation achieved at similar dosage in different species. Midazolam may have anaesthetic-sparing properties when used as a premedicant prior to inhalation anaesthesia. Oppenheim & Moon (1995) found that midazolam sedated *Trachemys scripta*, whereas Holz & Holz (1994) were unconvinced. Harvey-Clarke (1993) was unconvinced of sedative properties when midazolam was given IM to *Chrysemys picta* at doses as high as 20 mg/kg. Similarly in *Chelydra serpentina*, Bienzle *et al.* (1992) did not feel that the agent achieved useful sedation on its own.

Bienzle *et al.* (1992) compared the effects of ketamine, midazolam and combinations of these two agents in snapping turtles *Chelydra serpentina*. They concluded that combinations produced significant improvements over the use of single agents. Combinations of these drugs are discussed later in the section describing ketamine.

Holz & Holz (1994) did not find that surgical anaesthesia was achieved using 2 mg/kg midazolam and 60 mg/kg ketamine in *Trachemys scripta*. They found no improvement with this combination compared to ketamine on its own. This author would advise that midazolam is best combined with another agent such as ketamine if its use is considered in chelonians.

There is evidence that benzodiazepines inhibit metabolism of ketamine and potentiate its actions (Borondy & Gelazo 1977), however it is not clear how significant this is in clinical situations. Oppenheim & Moon (1995) suggest that the benzodiazepine antagonist flumazenil may shorten recovery time of animals sedated with midazolam or ketamine/midazolam. Table 14.2 below summarises the published work on anaesthesia using midazolam.

Zolazepam

Zolazepam is a minor diazepine tranquilliser, which is used mainly in combination with tiletamine (Telazol®, A.H. Robins; Zoletil®, Virbac). This drug combination is described below in Dissociative Anaesthetics.

Alpha-2 agonists

Xylazine

In *Trachemys scripta*, Holz & Holz (1994) used 2 mg/kg xylazine in combination with 60 mg/kg ketamine. However, the authors did not feel that the sedative effect was superior to 60 mg/kg ketamine on its own. This product is described later in Dissociative Anaesthetics.

Medetomidine

Norton *et al.* (1998) and Lock *et al.* (1998) describe the use of medetomidine in combination with ketamine in various chelonian species. This drug combination is described later in Dissociative Anaesthetics. Bennett (2000: personal communication) suggests the majority of the effect achieved with a medetomidine/ketamine combination is due to the medetomidine as so little ketamine is used. There is potential to reverse medetomidine using atipamezole (Table 14.3).

Opiates

Opiates appear to be ineffective in most reptiles and the reasons for this are poorly understood (Bennett 1995). Little hard data is known about opiates in chelonians and further studies are needed to clarify if, when and how their use may be appropriate.

Etorphine

Etorphine has been used in reptiles to induce anaesthesia, but its ability to cause the death of humans currently makes its use in chelonians unpopular.

Butorphanol

Bennett (1998a) reports that butorphanol (0.4 mg/kg IM) given 20 minutes before anaesthesia has been suggested to decrease induction agent requirements and provide sedation and analgesia.

Table 14.2 Literature describing the use of midazolam in chelonians.

Species (sample group)	Induction/duration/ recovery times	Midazolam dose and route	Comment	Reference
Chelydra serpentina	Not achieved	2.0 mg/kg IM	Inadequate sedation was achieved using midazolam sedation alone.	Bienzle *et al.* (1992)
Chrysemys scripta	Not achieved	20 mg/kg IM	The authors concluded this agent was ineffective in this species.	Harvey-Clarke (1993)
Trachemys scripta elegans (10 turtles were injected and compared with two turtles injected with water)	5.5 minutes induction (4–28 minutes range); 82 minutes duration (3–114 minute range); 40 minutes recovery (20–60 minute range)	1.5 mg/kg IM for sedation	Individual variations in response were observed, however the authors still suggest midazolam is safe and effective in this species.	Oppenheim & Moon (1995)

Table 14.3 Medetomidine and reversal with atipamezole.

Agent	Species	Dose and route	Induction time	Comment	Reference
Medetomidine	*Gopherus agassizii* (Ten tortoises)	150 µg/kg medetomidine IM	Sedation was achieved in all tortoises by 20 minutes post injection	Sedation was accompanied by a significant decrease in mean heart and respiratory rates, systolic, diastolic, and mean ventricular pressures, and mean ventricular partial pressure of oxygen (PO2).	Sleeman & Gaynor 2000
Atipamezole	*Gopherus agassizii* (five tortoises)	0.75 mg/kg IM	All tortoises normal within 30 minutes	Non-reversed animals remained sedated. Atipamezole will reverse the sedation but not all of the cardiopulmonary effects, thus necessitating continued monitoring after reversal.	Sleeman & Gaynor 2000

A combination of 0.4 mg/kg butorphanol with 2 mg/kg midazolam is also described. 0.2 mg/kg was suggested by Heard (1993) to sedate *Gopherus agassizii*. Malley (1999) suggests that up to 25 mg/kg can be used in tortoises for alleviation of pain.

Buprenorphine

Malley (1997) suggests 0.01 mg/kg IM as a postoperative analgesic.

Barbiturates

Barbiturates are of questionable value in reptiles as they generally result in prolonged and unpredictable induction and recovery times (Bennett 1991a & 1995). Therefore they cannot be routinely recommended as clinical anaesthetic agents. Barbiturates are widely used at high dosage for the humane euthanasia of reptiles and they serve this function well. Table 14.4 summarises the literature.

Dissociative anaesthetics

Phencyclidine, ketamine, and tiletamine have all been used successfully in reptiles (Bennett 1995). Their use is well described in the literature, making them useful agents in the anaesthesia of healthy specimens. However, when used in debilitated patients their apparent prolonged metabolism makes them unsuitable. Slow recoveries are potentially the result of slow absorption of massive intramuscular or intracoelomic doses. Little information is available on the use of small intravenous doses.

Dissociative anaesthetics produce a cataleptic state of rigidity without muscle relaxation. Little visceral analgesia is produced and they may therefore be unsuitable for intracoelomic surgery. This author seldom uses dissociative agents for anything other than improved handling during examination or administration of medications. They are however suggested as induction agents allowing intubation and maintenance with volatile inhalation agents by several authors, and moderate intravenous doses (e.g. 2–10 mg/kg ketamine) may facilitate intubation without the penalty of a prolonged recovery period.

In veterinary anaesthesia, ketamine and tiletamine are regularly used in combination with other drugs such as α–2 agonists, diazepam, midazolam and zolazepam. Some combinations are described below.

Ketamine (numerous preparations are available)

According to Bennett (1998a), ketamine is suitable for sedation or to allow intubation and maintenance with inhalation agents. Authors such as Bienzle *et al.* (1992) regard ketamine as only a moderately effective chemical immobilisation agent on its own. As the loss of ability to respond to pain in chelonians injected with ketamine is unpredictable, the advice of early literature suggesting that this agent is suitable to induce anaesthesia on its own is probably inaccurate.

Sedgwick (1988) gives scaled dosage rates for reptiles based upon body weight. Animals of 0.05–1 kg require 50 mg/kg and animals of 50–100 kg require only 8 mg/kg (patients between 1–50 kg falling somewhere in between!). However, such dosage regimens do not consider species differences or the degree of immobilisation required. Crane *et al.* (1980) unsuccessfully attempted immobilisation for intubation with 20 mg/kg IM in a 44 kg Galapagos tortoise (*Chelonoidis nigra*) injected in the proximal forelimb. From subsequent experience, these authors report 40–50 mg/kg to be more suitable.

Ketamine is painful by injection and is best given by deep intramuscular administration (Sedgwick 1988; Bennett 1998a). Bennett (1991a) reports that a single dose of 66 mg/kg may be adequate for three-day sedation of a reptile during transportation. Recovery from this agent is dose dependent and may be prolonged.

The use of moderate doses of intravenous ketamine (5–10 mg/kg), alone or in combination with other agents, may facilitate intubation and further induction maintenance with volatile agents (Norton *et al.* 1998; Lock *et al.* 1998). Such techniques do not yet appear to have been adequately explored. Table 14.5 summarises the literature concerning the use of ketamine in chelonian anaesthesia.

Tiletamine HCL and zolazepam (Telazol®, A.H. Robins; Zoletil®, Virbac)

In the United States, Telazol is a 1:1 combination of tiletamine and zolazepam. Literature reviewed by Bennett (1998a) suggests

Table 14.4 Literature describing the use of barbiturates in chelonians.

Agent	Species	Induction time	Dose and route	Comment	Reference
Sodium pentobarbitone	*Trachemys scripta*	30 minutes	15 mg/kg IC (intracoelomic)	All turtles retained a corneal reflex. 2.5 hours of surgical anaesthesia were produced.	Kaplan & Taylor (1957)
Chlorpromazine and sodium pentobarbitone	*Trachemys scripta*	5 minutes	10 mg/kg chlorpromazine IM followed by 10 mg/kg sodium pentobarbitone IC	All turtles retained a corneal reflex. Surgical anaesthesia lasted 2.9 hours. Recovery occurred in 4 hours.	Young & Kaplan (1960)
Sodium pentobarbitone	*Testudo graeca*	78 minutes (+/– 15 minutes)	18.2 mg/kg IC	2.5–4 hours anaesthesia	Hunt (1964)
Sodium pentobarbitone	'Tortoise'	Not specified	18.2 mg/kg IC	2–4 hours anaesthesia	Jones (1977)
Sodium pentobarbitone	*Chelonia mydas*	14–120 minutes	10–26 mg/kg slow IV induction	Injection was into the dorsal cervical sinus. Duration of surgical anaesthesia was 40–240 minutes. Recovery time was 4–24 hours. An initial dose is given at 10 mg/kg followed by 5 mg/kg supplementary doses every 15–30 minutes to a total cumulative dose of 25 mg/kg. The procedure was ineffective in 10% of cases.	Wood *et al.* (1982)
Sodium thiopentone	*Chelonia mydas*	5–10 minutes but unreliable	18.8–29.9 mg/kg IV	Duration of anaesthesia was 5–120 minutes. One turtle died at a dosage of 20 mg/kg and 10% of others failed to become anaesthetised at 31.4 mg/kg. Irritant in mammals when injected extravascularly.	Wood *et al.* (1983)
Sodium pentobarbitone	*Chelonia mydas*	Not specified	10–25 mg/kg IV induction	Recovery time was 4–24 hours.	Butler *et al.* (1984)

that tiletamine is significantly (2–3 times) more potent than ketamine. It acts synergistically with zolazepam to decrease muscle rigidity and seizures in mammals. Because patients remain sensitive to external stimulation even at high doses, it is inappropriate as a sole method of providing surgical anaesthesia and must therefore be combined with local anaesthesia or inhalation anaesthesia. This combination may also increase oronasal secretion. Schobert (1982) suggests that doxapram (Dopram®, Vétoquinol) may be of use if profound respiratory depression and overdose occur.

High doses of Telazol (>15 mg/kg) may result in prolonged sedation of 24–48 hours. If an adequate effect is not seen at doses approaching 10 mg/kg, consider supplementing with low, incremental doses of a second agent such as propofol (2–5 mg/kg IV) or medetomidine (50 µg/kg IM). Alternatively, a reversible neuromuscular blocking agent, rocuronium (0.25–0.5 mg/kg IM) (750 g turtle), reversed by neostigmine (0.04 mg/kg IM) or glycopyrrolate (0.01 mg/kg IM) may be a useful addition for non-painful procedures (Kaufman *et al.* 2001) (Table 14.6).

Steroid anaesthetics

Alphaxalone/alphadolone (Saffan®, Schering-Plough Animal Health)

Alphaxalone/alphadolone is presently unavailable in North America. Induction time after IV injection is rapid and may be as short as 30 seconds. Anaesthesia is generally smooth and uneventful. Recovery at 10–20 minutes post IV injection tends to be reliable. Repeat doses for maintenance result in predictable extension of anaesthesia. Violent shaking and twitching have been described in reptiles during recovery from intramuscular injection (Lawrence & Jackson 1983a), but such signs are seldom encountered with IV administration.

This author (SM) has used IV alphaxalone/alphadolone routinely in chelonians for over ten years and, at the time of writing, it remains his induction agent of choice. Intramuscular injection as suggested by Lawrence & Jackson (1983) appears unreliable in chelonians (Harper 1984; McArthur 1996) and is therefore not encouraged. Occasional reports in the literature suggest that

Table 14.5 Literature describing the use of ketamine in chelonians.

Agent	Species	Dose and route	Induction time	Comment	Reference
Ketamine	*Testudo graeca* *Trachemys scripta*	25–65 mg/kg for handling	10–15 minutes with higher dose rates	Recovery of 4–24 hours is reported.	Jones (1977)
Ketamine	*Chelonoidis nigra*	20 mg/kg IM was insufficient to allow intubation of a 44 kg tortoise. The authors report that 40–50 mg/kg was subsequently deemed to be more suitable.	Not specified	Induction agent allowing halothane inhalation to be used for maintenance.	Crane *et al.* (1980).
Ketamine	*Chelonia mydas*	50–71 mg/kg IC over 25 minutes	2–10 minutes after final administration	Surgical anaesthesia lasted 2–10 minutes. Recovery took 4 hours.	Wood *et al.* (1983)
Ketamine	*Chelonia mydas*	50 mg/kg IM	Not specified	Unsuccessful at inducing surgical anaesthesia on its own.	Wood *et al.* (1983)
Ketamine	Turtles/tortoises	Sedation 40–60 mg/kg IM; anaesthesia 60–90 mg/kg IM	30 minutes	Poor muscle relaxation; long recovery.	Johnson (1991)
Ketamine	Reptile (species not specified)	Sedation 22–44 mg/kg IM. Surgical anaesthesia 55–88 mg/kg. Doses above 110 mg/kg often cause respiratory arrest and bradycardia.	10–30 minutes	Recovery takes 24–96 hours.	Bennett (1998a)
Ketamine	*Trachemys scripta elegans*	60 mg/kg IM	15–24 minutes—if at all!	Inadequate for surgery but may be suitable for immobilisation.	Holz & Holz (1994)
Ketamine and xylazine	*Trachemys scripta elegans*	Ketamine 60 mg/kg IM; xylazine 2 mg/kg IM	11–35 minutes—if at all!	Inadequate for surgery but may be suitable for immobilisation. The drug combination was no improvement over ketamine alone.	Holz & Holz (1994)
Ketamine and midazolam	*Trachemys scripta elegans*	Ketamine 60 mg/kg IM; midazolam 2 mg/kg IM	7–47 minutes—if at all!	Was considered unsuitable for surgery but may be suitable for immobilisation. Was no improvement over ketamine alone.	Holz & Holz (1994)
Ketamine	*Chelydra serpentina*	Ketamine 20–40 mg/kg IM	Within 5 minutes	Inadequate for surgery but may be suitable for immobilisation.	Bienzle *et al.* (1992)
Ketamine and midazolam	*Chelydra serpentina*	Ketamine 20 mg/kg; midazolam 2 mg/kg IM	Within 5 minutes	Inadequate for surgery but may be suitable for immobilisation.	Bienzle *et al.* (1992)
Ketamine and midazolam	*Chelydra serpentina*	Ketamine 40 mg/kg; midazolam 2 mg/kg IM	Within 5 minutes	Inadequate for surgery but may be suitable for immobilisation. The authors felt this was better than the 20 mg/2 mg combination.	Bienzle *et al.* (1992)
Ketamine and medetomidine	*Gopherus polyphemus*	75 µg/kg medetomidine and 7.5 mg ketamine IV	19.8 minutes (mean time to stage 1 anaesthesia)	All animals could be intubated and reached stage 3 anaesthesia. Reversal with atipamezole at five times the medetomidine dosage reduced recovery time.	Norton *et al.* (1998)
Ketamine and medetomidine	*Gopherus polyphemus*	50 µg/kg medetomidine and 5 mg/kg ketamine IV	15 minutes (mean time to stage 1 anaesthesia)	This combination provided stage 3 anaesthesia in 74% of tortoises. Reversal with atipamezole at five times the medetomidine dosage reduced recovery time.	Norton *et al.* (1998)

Table 14.5 (*cont'd*)

Agent	Species	Dose and route	Induction time	Comment	Reference
Ketamine and medetomidine	*Geochelone pardalis* *Geochelone denticulata*	100 µg/kg IV medetomidine 5 mg/kg ketamine IV	4–16 minutes	Reversal with atipamezole at 400 µg/kg gave recovery in 5 minutes.	Lock *et al.* (1998)
Ketamine and medetomidine	*Dipsochelys elephantina*	25–80 µg/kg IV medetomidine; 3–8 mg/kg ketamine IV	15–45 minutes	Reversal with atipamezole at 100–380 µg/kg (4 times the dose of medetomidine) gave 5–15 minutes for recovery.	Lock *et al.* (1998)
Ketamine and ACP	Sea turtles	For 10 kg turtles, 30 mg/kg of ketamine solution mixed with 10% by volume of acepromazine was used: • For 50 kg turtles 19.9 mg/kg • For 150 kg turtles 15.2 mg/kg • For 400 kg turtles 11.8 mg/kg (ketamine). It was suggested that leatherback turtles required 15% more than other species.	Not specified	A cocktail of 10 ml ketamine to 1 ml acepromazine (ketamine HCL 100 mg/ml; acepromazine maleate 10 mg/ml) caused a significant reduction in the dose of ketamine required to intubate and ventilate turtles with volatile agents. George (1997), and Whittaker & Krum (1999), suggest this to be the induction method of choice in marine turtles in view of the long recovery times described by Moon & Stabenau (1996) using forced intubation and inhalation induction.	George (1997), Whittaker & Krum (1999)

Table 14.6 Literature describing the use of tiletamine and zolazepam in chelonians.

Species	Induction time	Dose and route	Comment	Reference
Terrapene carolina triunguis	15 minutes to effect but anaesthesia not achieved.	4.4–33 mg/kg IM	Sedation was similar at all doses. Surgical anaesthesia was never achieved. Intubation and inhalation anaesthesia were potentially possible. Recovery was 1–1.75 hours.	Boever & Caputo (1982)
Terrapene carolina triunguis	9 minutes to effect but anaesthesia not achieved.	44–88 mg/kg IM (probable overdose)	Sedation was similar at all doses. Surgical anaesthesia was never achieved. Intubation and inhalation anaesthesia were potentially possible. Recovery was 11 hours, therefore lower doses were advised by the authors.	Boever & Caputo (1982)
Chelonia mydas	Not specified	15 mg/kg IM	One animal died 10 hours post-anaesthesia, another was intubated and maintained using isoflurane.	Jacobson *et al.* (1991a)
Trachemys scripta	Not specified	3.5–14 mg/kg IM	Limited data presented	Schobert (1982)
Clemmys insculpta	Not specified	10 mg/kg IM	Limited data presented	Schobert (1982)
Turtles/tortoises	>20 minutes	3–10 mg/kg IM	Poor muscle relaxation	Johnson (1991)
Trachemys scripta elegans	Intubation was possible15 minutes later.	2 mg/kg IM	Anaesthesia was maintained with halothane following intubation.	Gould *et al.* (1992)
Chelonian	Not specified	10–20 mg/kg IM	Sedation to allow intubation and maintenance with a volatile agent.	Page (1993)

Table 14.7 Literature describing the use of alphaxalone/alphadolone in chelonians.

Species	Induction time	Dose and route	Comment	Reference
Gopherus polyphemus	Not specified	9 mg/kg IC (intracoelomically)	Used for sedation	Calderwood (1971)
Testudo graeca; Testudo hermanni; Trachemys scripta	25–40 minutes	9–16 mg/kg IM	15 mg/kg is recommended for up to 20 minutes surgical anaesthesia. Two tortoises were shaky in recovery.	Lawrence & Jackson (1983)
Large patients (not specified)	15 minutes	9–12 mg/kg IV	Recovery ~4 hours	Harper (1984)
Testudo hermanni; Testudo horsfieldi; Testudo graeca; Testudo marginata; Geochelone pardalis; Pseudemys scripta; Geochelone radiata; Geochelone carbonaria	0.5–2 minutes	9–12 mg/kg IV	Results in an excellent plane of surgical anaesthesia that lasts for 10 or more minutes. Currently this author's (SM) drug of choice for anaesthetic induction. This also allows intubation and maintenance with a volatile anaesthetic agent.	McArthur (1996); McArthur (personal observation)

alphaxalone/alphadolone may have anti-analgesic qualities in some patients. No data is available for marine turtles, where this agent may prove to be of use in view of the short induction and recovery times recorded in terrestrial species (Table 14.7).

Propofol (Rapinovet®, Schering-Plough; Diprivan®, Zeneca)

Propofol is a hypnotic sedative that provides rapid induction. It is available as an emulsion and is not associated with perivascular irritation or inflammation. Once opened, it must be used immediately, as it is a lipid emulsion that lacks a preservative, and so is an ideal bacterial substrate. The manufacturer advises that unused portions should be discarded immediately. Recovery in chelonians appears to be variable and potentially related to primary dose, further incremental doses and speed of administration. Divers (1996a) suggests that typical induction times in chelonians are less than a minute unless perivascular injection occurs. Recovery in reptiles is typically 25–40 minutes (Divers 1996a).

In all species toxicity would seem to be low (Bennett 1998a). Apnoea and slow recovery, in comparison with other agents such as alphaxalone/alphadolone (Saffan®, Schering-Plough Animal Health), are described in chelonians, especially if the agent is 'topped up' during the course of anaesthesia (McArthur 1996). However, this comparison has not yet been appropriately investigated, and may not prove to be significant.

Bennett (1998c) demonstrated substantial hypoventilation, hypoxemia, hypercapnia and bradycardia in the green iguana, at 1 mg/kg/min. This author has observed similar effects in chelonians, particularly if a rapid intravenous bolus had been administered. Slow administration over 1–2 minutes seems to reduce this.

Following propofol induction, this author (SM) advises against using it for maintenance, preferring intubation and maintenance with a volatile agent such as isoflurane. Divers (1996a) suggests that incremental doses still provide acceptable recovery times in reptiles, but agrees that intubation and maintenance with a volatile agent such as isoflurane is the preferred practice (Table 14.8).

Neuromuscular blocking agents

Succinylcholine/suxamethonium chloride

The depolarising neuromuscular blocking agent succinylcholine chloride has been used to restrain chelonians. There are differing opinions as to its reliability, safety, efficacy, humanity and suitability. No analgesia or hypnosis is produced. It is *not* an anaesthetic. However, immobilisation may allow intubation and maintenance with analgesia through ventilation with volatile agents. Depolarising agents affect a large number of muscles all over the body and in humans the experience of generalised depolarisation is extremely painful. It seems likely that the effect in chelonians will be similar. This author is unable to find any indications for the use of this agent.

The use of succinylcholine chloride in chelonians has been described by Page & Mautino (1990) at 0.25–1.5 mg/kg. Surgical use involves combination with local or gaseous anaesthesia. It is very hard to monitor sensation and analgesia. Intramuscular injection is essential because it is absorbed ineffectively if administered into fat. This type of drug should not be used in combination with drugs such as aminoglycosides. Boyer (1992a) suggests that incremental dosing with this agent is likely to precipitate fatalities in chelonians. Sudden death has occurred in an apparently healthy spurred tortoise (*Geochelone sulcata*) given succinylcholine chloride at 0.5 mg/kg IM (Jessop 1999: personal communication). Similar comments were made at the Association of Reptilian and Amphibian Veterinarians question and answer session in Columbus, Ohio (1999). This agent sometimes causes severe hyperkalaemia. Prolonged paralysis is possible in ill patients; liver disease, dehydration and electrolyte imbalance are all possible (Hale 2000: personal communication).

Four seemingly healthy *Geochelone pardalis* restrained by this author (SM) using succinylcholine chloride at a similar dosage were difficult to manipulate due to residual muscle tone. This author has also observed prolonged recovery times with succinylcholine chloride despite suitable temperature and fluid provision, and is concerned that animals immobilised in this manner

Table 14.8 Literature describing the use of propofol in chelonians.

Species	Induction time	Dose and route	Comment	Reference
Chelonians	Less than a minute	12–14 mg/kg IV	Partial perivascular injection may result in prolonged induction time. Intubation and maintenance with isoflurane advised as usual practice.	Divers (1996)
Testudo graeca	Not specified	14 mg/kg IV	Allowed intubation and maintenance with isoflurane anaesthesia.	Lawton (1996)
Chelonians	1–4 minutes	10 mg/kg IV for induction	Dorsal tail vein	Bennett (1998a)
Giant chelonians	Not specified	2 mg/kg IV for induction	Catheterised jugular vein	Bennett (1998a)
Tortoise	Not specified	5–14 mg/kg IV, IO (intraosseous)	Suggested to work well for short procedures.	Divers (1998c)
Testudo graeca	Not specified	10–14 mg/kg IV	Allowed intubation and maintenance using isoflurane.	Divers (1998c)
Red-eared sliders		15–30 mg/kg IO		Fonda (1999)

Table 14.9 Literature describing the use of succinylcholine chloride in chelonians.

Species	Induction time	Dose and route	Comment	Reference
Turtles/tortoises	20–30 minutes	0.5–1 mg/kg	No analgesia. Immobilisation agent only. Allows intubation and maintenance with analgesia through ventilation with volatile agents.	Johnson (1991)
Chelonian	20 minutes	0.25–1.5 mg/kg IM	Recovery suggested to take 45 minutes. Ventilation is occasionally required.	Page & Mautino (1990)
Large chelonians, marine turtles	20–30 minutes	0.5–1.0 mg/kg IM	Recovery takes 2–3 hours. Respiration is usually maintained.	Bennett (1998a)

are distressed. Ventilation with a volatile agent (such as 1%–2% isoflurane) may improve patient analgesia (Table 14.9).

Rocuronium (Zemuron®, 10 mg/ml, Organon Inc., West Orange, NJ)

Rocuronium provides neuromuscular blockade only and therefore complications described earlier with respect to succinylcholine/suxamethonium chloride apply. This agent must *never* be used alone to restrain animals for painful or stressful procedures. Kaufman *et al.* (2001) advocate the use of rocuronium to facilitate intubation and induction of anaesthesia with a gas inhalant (e.g. isoflurane). Every effort must be made to minimise distress during use prior to onset of full anaesthesia, including minimising extraneous noise, bright lights and physical manipulation.

Kaufman *et al.* (2001) state that reversal of rocuronium prior to the start of the surgical procedure is essential, so that the effects of the anaesthesia and presence of adequate analgesia may be evaluated accurately. Reversal is readily achieved with intramuscular glycopyrrolate and neostigmine at doses of 0.01 mg/kg and 0.04 mg/kg respectively (Boyer 1992; Lloyd 1994), given 30 minutes after rocuronium injection (Table 14.10).

GASEOUS AGENTS

In tortoises and turtles, intubation can be difficult or even impossible without the use of injectable immobilising or relaxing agents such as alphaxalone/alphadolone, propofol, ketamine and tiletamine/zolazepam and muscle relaxants such as succinylcholine chloride. All are described earlier. Following immobilisation, intubation of chelonians is generally quite easy. Topical anaesthetic (lignocaine HCL 1%–2%) can be applied to the glottis with a cotton swab to facilitate intubation.

Isoflurane

Isoflurane undergoes extremely limited renal or hepatic excretion. It is excreted almost exclusively by the lungs and is appropriate for use in debilitated patients (Bennett 1995). However, it is possible that the respiratory excretion of isoflurane has negative influences on recovery time in species capable of cardiovascular shunting away from the respiratory system. Excretion of inhalation agents such as isoflurane in diving chelonians has not yet been investigated. The agent may have unexpectedly long recovery times in some reptiles such as chelonians, when compared to

Table 14.10 Literature describing the use of rocuronium in chelonians.

Species	Induction time	Dose and route	Comment	Reference
Terrapene carolina major (approximately 750 g)	Just less than 10 minutes	0.25–0.5 mg/kg IM	Intubation and maintenance with inhalant agents advised as usual practice. Reversal of the neuromuscular blockade advised at the start of any procedure requiring analgesia.	Kaufman *et al.* (2001)
Reptiles (substantially greater or less than 750 g)	Just less than 10 minutes	Recommended to be used following allometric scaling techniques (0.02–0.05 mg/kcal).		Kaufman *et al.* (2001)

Table 14.11 Literature describing the use of isoflurane in chelonians.

Species	Induction time	Dose and route	Comment	Reference
Reptile (not specified)	1–5 minutes	3% induction; 1.5% maintenance	This author's (SM) volatile anaesthetic of choice. Anaesthetic recovery can take up to 3 hours.	Johnson (1991)
Reptile (not specified)	6–20 minutes	4%–5% induction; 1.5%–4% maintenance	The author's agent of choice in debilitated reptiles.	Bennett (1995)
Lepidochelys kempii	7 +/– 1 minutes	3%–4% induction; 2.5%–3% maintenance	Induction achieved by forced intubation and ventilation. For an anaesthetic duration of 131 minutes, the recovery time was 241 minutes. Cardiovascular shunting away from pulmonary vasculature was proposed as a reason for prolonged recovery.	Moon & Stabenau (1996)
Chelydra serpentina	Not achieved	5%	Turtles were placed in 5% isoflurane for 90 minutes but did not become anaesthetised.	Bienzle *et al.* (1992)
Chelonia mydas	Not specified	Not specified	Recovery took 2–6 hours.	Shaw *et al.* (1992)

Table 14.12 Literature describing sevoflurane use in chelonians.

Species	Induction time	Dose and route	Comment	Reference
Gopherus agassizii	2.5 +/– 0.55 minutes	Intubation and forced ventilation	Recovery time was 127.58 +/– 7.55 minutes and duration of anaesthesia was 105 +/– 12 minutes. The authors conclude this was a safe and effective anaesthetic agent, providing rapid induction and recovery.	Rooney *et al.* (1999)

its use in mammalian species, notably where conditions favour intrapulmonary shunting and so decrease its excretion (Table 14.11).

Sevoflurane

Six desert tortoises (*Gopherus agassizii*) were intubated whilst awake and ventilated manually with 3%–7% sevoflurane and oxygen (Rooney *et al.* 1999). Mader (1999: personal communication) comments that sevoflurane is similar in most properties to isoflurane, however its reduced smell may make it more suited to inhalation induction (Table 14.12). Recent studies at the University of Georgia have also suggested a significant reduction in recovery time with sevoflurane.

Halothane

One study exposed box turtles (*Terrapene carolina* and *Terrapene ornata*), red-eared sliders (*Trachemys scripta*) and snapping turtles (*Chelydra serpentina*) to high concentrations of halothane for 60–90 minutes in a sealed container. Anaesthesia was not produced, as a result of prolonged breath holding (Brannian *et al.* 1987). Similar exposure of *Kinosternon* spp. resulted in induction because these turtles failed to breath hold, possibly for behavioural reasons.

This agent is suitable for use as an anaesthetic maintenance agent following IV-agent induction. Whilst its use is currently less popular than isoflurane there is a suggestion from the data

Table 14.13 Literature describing the use of halothane in chelonians.

Species	Induction time	Dose and route	Comment	Reference
Kinosternon spp.	5–35 minutes	Open drop inhalation	Recovery took 5–15 minutes	Brannian *et al.* (1987)
Aldabran tortoise	N/A	1%–2% maintenance	None	Crane *et al.* (1980)
Reptile	N/A	1.5% maintenance	Recovery can take up to 7 hours	Johnson (1991)
Reptile	N/A	2–3% maintenance	A recovery time of 5–60 minutes was reported.	Butler *et al.* (1984)

above that it might occasionally produce a more rapid recovery (Table 14.13).

Methoxyflurane

Methoxyflurane is rarely used because of its slow induction time, prolonged recovery time and potential nephrotoxicity. Anaesthetic recycling may also occur with this agent (Bennett 1998a).

Nitrous oxide

Nitrous oxide can be used up to a 2:1 mixture with oxygen and is thought to have analgesic qualities in reptiles. A ten-minute period of re-oxygenation (ventilation with oxygen or air to flush out residual nitrous oxide) is wise before disconnection from the anaesthetic circuit, in order to prevent diffusion hypoxia. The effect of nitrous oxide/oxygen mixtures on cardiovascular shunting is unknown, as are toxicity effects.

PATIENT RECOVERY

Recovery should be in a calm environment at the upper end of the animal's ATR and at a suitable humidity. Fluid administration may increase the metabolism and renal excretion of some agents. Warmth may increase the metabolic rate and so increase metabolism and excretion of anaesthetic agents. However, excessive warmth may be harmful, as tissue oxygen demands may increase (Bennett 1991a, 1995 & 1998a). Recovery from most agents takes longer than in mammals (Bennett 1991a). Cardiovascular shunting and ventilation-perfusion mismatch have already been described.

At this author's (SM) clinic, chelonians remain intubated during the recovery phase and manual or automatic ventilation with air or oxygen is continued until voluntary movements are being made and a righting reflex is evident. Limb movements aid ventilation and the passage of air through the airways is easy to hear if the head and limbs are gently pumped (Figs 14.21–14.22). Occasional animals will resume spontaneous breathing and then relapse as recovery proceeds. It is best to maintain endotracheal intubation until the recovery is complete and the patient seems alert (Bennett 1998a).

Respiratory stimulants

According to Boyer (1992a), Malley (1997) and Bennett (1998a), doxapram (5 mg/kg IM or IV every 10 minutes) may be used to stimulate respiration.

ANALGESIA

Pain and analgesia are poorly understood in reptiles. Whilst the sedative effects of opiates in most reptiles have not been recorded, it is suggested that opiate receptors present in reptiles do modulate pain. Non-steroidal anti-inflammatory drugs (NSAIDs) are utilised by many veterinarians to alleviate peri-operative pain in chelonians. Normal feeding behaviour and activity are given as anecdotal evidence of efficacy. Carprofen and butorphanol appear to be the current agents of choice. Malley (1997) points out that adequate renal function should be confirmed prior to the use of non-steroidal anti-inflammatory drugs. Carprofen has hepatotoxic effects in mammals and should only be used cautiously for a short time, if at all, in chelonians with evidence of hepatic or renal damage/disease (Table 14.14).

EUTHANASIA

Indications for euthanasia include the humanitarian prevention of unnecessary suffering, failure to find a suitable captive environment for an unwanted chelonian, preparation for experimental work or post-mortem examination or, in the case of farmed turtles, slaughter for human consumption (Jackson & Cooper 1981a; Johnson 1991; McArthur 1996; Jacobson *et al.* 1999a). Different situations require different methods. Animals for human consumption are generally managed differently from captive pets undergoing euthanasia in the company of their keeper. Whatever method is employed the animal should be exposed to the minimum pain, trauma and distress possible during the procedure.

Evidence suggests that biochemical and electrical activity persists within an anoxic turtle brain for some considerable time (Cooper *et al.* 1984; Nilsson & Lutz 1991; Fernandez *et al.* 1997; Lutz & Manuel 1999). This means that euthanasia with some agents could result in unexpected recovery due to the ability of chelonian brains to survive prolonged anoxia. For chelonian patients, therefore, this author advises pithing, or brainstem injection of formalin or local anaesthetic solution in combination with a lethal injection.

It seems logical to hypothesise that euthanasia may be best performed using a high dose of a cardioplegic agent, such as lignocaine, K^+ or Mg^{2+} salt, as opposed to an anaesthetic agent unless immediate pithing is performed. In order to prevent survival, it is necessary to prevent the possibility of recovery following metabolism of barbiturate or other anaesthetic agent. Apnoea alone may not be sufficient to precipitate death. *Trachemys scripta*

Table 14.14 Analgesic agents for chelonians.

Agent	Doses	Comments
Butorphanol	• Bennett (1998a) reports that butorphanol (0.4 mg/kg IM) given 20 minutes before anaesthesia decreases induction-agent requirements and provides sedation and analgesia. A combination of 0.4 mg/kg with 2 mg/kg midazolam is also described. • 0.2 mg/kg to sedate *Gopherus agassizii* was suggested by Heard (1993). • 0.05 mg/kg every 24 hours for 2–3 days has been used by some American colleagues.	Variation in dose rate and limited data regarding efficacy suggest that further research is required to determine the efficacy and pharmacodynamics of this product in chelonians.
Carprofen	2–4 mg/kg IM, IV, SC or orally, followed by 1–2 mg/kg every 24–72 hours (Malley 1997; Divers 2000: personal communication).	This product has been used peri-operatively by this author during surgical procedures such as coeliotomy and ear abscess drainage. Subjectively it appears to reduce peri-operative pain. Where renal function is considered adequate, it appears to be the NSAID of choice in chelonians, given that we have no experience of other prostaglandin-sparing NSAIDs in chelonians.
Buprenorphine	0.01 mg/kg IM is suggested by Malley (1997) as a postoperative analgesic.	

elegans has been shown to survive up to 27 hours in a 100% nitrogen environment (Johlin & Moreland 1933).

Pithing may not be possible if further examination of the brain is required. In such a case intracranial injection of formalin through the foramen magnum after decapitation has been advised (Frye 1991a). Unfortunately, if the brain is intended for microbiology or virus isolation neither procedure above can be considered practical. In such cases it may be best to remove the central nervous system from the cranial vault. In small chelonians pithing is easily performed through the roof the mouth using a dental probe. In large chelonians it may be necessary to precede pithing with decapitation. A captive-bolt humane killer or free bullet may even be required (Figs 14.25–14.29).

Frye (1991a) points out that the reptilian heart often beats for some considerable time following euthanasia and he suggests that this may allow collection of blood for further investigation.

Fig. 14.25 Equipment required for chelonian euthanasia is relatively simple and available in most veterinary surgeries. Injectable ketamine and phenobarbitone, a lengthy needle capable of intracardiac injection through the cranial carapacial inlet and a dental spike suitable for pithing are illustrated.

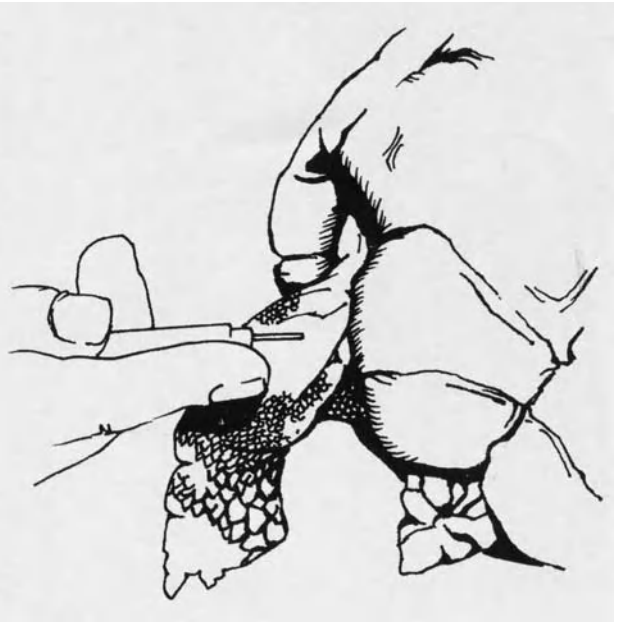

Fig. 14.26 Euthanasia (1): In order to facilitate handling and decrease animal distress, a large enough dose of ketamine to induce general anaesthesia is injected intramuscularly. Within a few minutes the animal is induced into a comfortable and pain-free state acceptable to owners who may be observing this procedure.

METHODS OF EUTHANASIA

Lethal injection (combination method)

Pre-medication

Pre-medication with ketamine facilitates intravenous injections in chelonians and active animals and makes the whole situation far less distressing for both the patient and any keeper who may be present.

Fig. 14.27 Euthanasia (2): In the now ketamine-anaesthetised animal, concentrated phenobarbitone solution marketed for small animal euthanasia is injected into the heart through the cranial carapacial inlet. A 19G, 40 mm needle on a 5 ml syringe is ideal for most moderately-sized animals (less than 5 kg).

Fig. 14.28 Euthanasia (3): Pithing through the roof of the mouth is advisable for animals which are to return home with their keepers/owners following euthanasia. A curved dental sulcus-cleaning spike is easily inserted into the cranial vault and rotated to destroy the brainstem.

This author (SM) gives a pre-medication dose of ketamine IM (100–200 mg/kg) and follows this with an intravenous injection of 200 mg/kg pentobarbitone solution and pithing. This system has proven consistently effective and is currently this author's method of choice.

Intracardiac injection

Intracardiac injection can often be achieved by a cranial approach parallel to the neck, directed towards the midline. Intravenous injection into the jugular, subcarapacial or dorsal tail vein is usually simple following the earlier injection of ketamine.

Fig. 14.29 Euthanasia (4): Here the animal is pithed by insertion of a spike through the foramen magnum. The spike is then rotated to destroy the brainstem.

Intracranial injection

Barbiturate injection through the foramen magnum may speed death and reduce movements associated with pitting (SM personal observation).

Other methods

Intracoelomic injection

Intracoelomic injection of chloroform has been advised in early texts such as Jackson & Cooper & Jackson (1981a) but this may be inhumanely painful in comparison to the combination method advised earlier and so this method is not advised. Intracoelomic injection of other agents is not advised, as the time to death may be prolonged when compared to the combination method.

Inhalation

Inhalation of volatile agents carbon dioxide and nitrous oxide are ineffective in most chelonians due to their remarkable ability to breath hold. This method is not advised.

Drugless methods

Electrical stunning, exsanguination, decapitation and pithing may be appropriate in field conditions where drugs and facilities are limited, or where slaughter for human consumption is anticipated. Contamination of the body by drug residues must be avoided if the animal is to be eaten.

Freezing

Freezing of reptiles as a method of euthanasia is not advised. There is evidence from sub-zero post-hibernation damage observed in tortoises, that painful brain and eye damage may

occur prior to death from freezing. Even as a method of immobilisation prior to the employment of other methods, the operator should remember that a chilled reptile might experience pain and distress if handled inappropriately.

Freezing may be appropriate after lethal injection and CNS injection to leave no doubt of death.

DIAGNOSING DEATH

This author regards death as the point at which recovery of brain activity is not possible. Therefore, a chelonian heart that continues to beat for many hours outside of a pithed animal, in which brain function has been destroyed, should not be misconstrued as an indication of the continuation of life! The following criteria are given as a guide:

- absence of detectable pulse or heartbeat using Doppler ultrasonography, oesophageal stethoscopy, ultrasonography, ECG or pulse oximetry;
- absence of unaided respiration and its failure to return despite appropriate ventilation attempts with oxygen or air;
- absence of reflex responses to external stimulus over several hours (corneal and cloacal reflexes are maintained until very near death, even in hibernating animals);
- absence of response to warming and warm fluid administration if the animal may be chilled or hibernating;
- tissue changes such as rigor mortis, grey/cyanotic mucous membrane colour, sunken and deflated eyes and post-mortem tissue necrosis;
- absence of brain activity, or a reduction in brain activity to a point where recovery is impossible.

Death would therefore appear to be a subjective diagnosis to a general practitioner.

SURGERY

STUART MCARTHUR, STEPHEN HERNANDEZ-DIVERS

With our ability to stabilise and manage debilitated chelonians improving dramatically year by year, clinicians are now able to perform a wide variety of surgical procedures (Table 15.1).

Chelonian patients differ from mammalian patients in many ways, both anatomical and physiological (Table 15.2).

Table 15.1 Range of surgical procedures now performed on chelonians.

Supportive techniques	• Placement of cardiac port • Placement of vascular access port • Placement of oesophagostomy tube
Lesion management	• Management of stomatitis • Skin abscess/fibriscesses • Ear abscess • Mandibular trauma management • Scute debridement in shell infections
Diagnostic techniques	• Endoscopy • Skin/shell/lesion biopsy
Trauma management	• Eye enucleation • Repair of traumatic injuries, e.g. shell trauma repair • Wheel application
Limb surgery	• Fractures • Trauma • Amputation • Management of osteomyelitis • Articular gout
Respiratory tract	• Carapacial access • Prefemoral access
Cloacal organ prolapse	• Reduction and retention • Amputation of traumatised penis • Amputation of prolapsed cloacal material • Amputation of prolapsed uterine material • Bladder prolapse reduction • Cloacal puncture and aspiration of impacted egg
Coeliotomy	• General visceral surgery • Removal of cystic calculi • Correction of egg retention • Ovariectomy • Retrieval of foreign bodies • Management of coelomitis • Prolapse reduction

PRE-OPERATIVE PATIENT PREPARATION

It is crucial that patients are stabilised appropriately prior to attempting surgical procedures, particularly when elective. Where an animal is committed to hibernation, anabolic repair processes will not be optimum for patient recovery. It is best to warm, hydrate and otherwise stabilise such cases unless immediate procedures are performed as life saving measures, this may take a week or two.

Anaesthesia and analgesia

The authors do not suggest forcibly restraining a chelonian and operating in the absence of analgesia. In most countries this is illegal. Appropriate agent(s) will depend on the type and duration of the procedure. Injectable analgesics, inhalation agents, and local anaesthesia by regional infiltration all have a place.

Antibiotics

Chelonians are best considered to be heavily faecally contaminated across their surfaces. Many will be immunocompromised or even septicaemic. Therefore, creating a sterile field for most chelonian surgical procedures is often difficult and pre-operative antibiotics should be considered.

This author routinely uses pre-operative ceftazidime or enrofloxacin at doses described in the Therapeutics chapter.

Where infection is present, choice of antibiotic is best determined by culture and sensitivity patterns.

Fluid management

It is desirable to maintain a reasonable level of fluid input and output in order to facilitate excretion of metabolic waste products and therapeutic agents. In particular, this will reduce the potential for gouty mineralisation of soft tissues. Unfortunately, to date a method for objective assessment of the hydration status of chelonians remains elusive. Both clinical and clinicopathological parameters have proved difficult to interpret. Hospitalisation with monitoring of the frequency and volume of urine output and urine specific gravity in relation to fluid administration is helpful when dehydration is suspected.

The identification of hypoperfusion ('shock') also presents problems. To our knowledge, physical signs of hypoperfusion have yet to be described in chelonians, but its existence must be assumed where significant blood loss or severe sepsis are identified.

Fluid management is covered in further detail in Anatomy and Physiology, Therapeutics, Clinical Pathology and Anaesthesia.

Table 15.2 Special considerations for surgery on chelonians.

Ossified dermal plates of the carapace and plastron	• The skin of most chelonians is composed of scales and scutes. • Scales are keratinised plates, often arranged in rows. • Scutes are the largest scales and often overlie bony dermal plates. • Soft-shelled and leatherback turtles do not have scales but have a leathery skin instead. • Most texts advise incisions between scales and scutes wherever possible (Millichamp 1988; Lawton & Stoakes 1992; Bennet 1993a; Mader 1996a). • The dermal plates of the carapace and plastron restrict access to viscera in smaller specimens. A plastron osteotomy is necessary in these circumstances. This procedure will require appropriate equipment (Millichamp 1988, Lawton & Stoakes 1992, Bennett 1993; Bennett & Mader 1996).
Limited access to free skin	• The skin of most chelonians is only mobile in areas where the carapace and plastron are absent. At the head and distal limbs, skin mobility is greatly reduced. Consequently, there are limitations on free skin available to cover dermal defects. • This author has used advancement and rotational flaps when removing skin lesions close to the head or shell. Despite its complexity, the outcome of such dermoplasty is generally good. Other options include leaving difficult wounds open to heal by second intention or using materials, such as Biosist®.
Stability and health of the patient	• Where elective surgery is performed, it is advisable to ensure that the patient is not in an anorexic and/or catabolic state. Chelonians in the United Kingdom are often presented at veterinarians poorly-maintained, malnourished, cold and chronically ill. This significantly influences post-operative recovery and wound healing. • Stabilisation of chelonian patients through hospitalisation within an optimal therapeutic environment (where temperature, light exposure and humidity suit the patient, and an organised fluid therapy protocol and nutritional support programme are applied), often improves surgical results in compromised patients. • Where biochemical imbalances, such as hyperkalaemia and hypoglycaemia, are likely, anaesthesia and surgery are best delayed until they have been corrected. This is discussed further in Clinical Evaluation. • Hospitalisation of critically-ill chelonians is discussed elsewhere in this text.
Ventral abdominal veins	• Two paired veins lie on either side of the midline below the plastron. They are found in the coelomic membrane and fascia, approximately midway between the midline and the bridge (where carapace and plastron join) (Fig. 15.1), and are incorporated into the coelomic membrane immediately dorsal to the bony plates of the plastron. They can usually be blunt dissected and exposed cleanly during a plastron osteotomy and coeliotomy. • Several authors and personal experience suggest that most patients are unaffected if these vessels are ligated as a haemostatic measure in case of inadvertent trauma. However, it is often easy to reflect them laterally without trauma, allowing an incision through the coelomic membrane closer to the midline. • If they are inadvertently traumatised, it is unwise to leave these vessels unligated, as continued haemorrhage may threaten the recovery of the patient. The potential for post-operative haemorrhage may not be apparent at the time of surgery.
Bladder and pericardium	• Both the bladder caudally and the pericardial sac cranially are continuous with the coelomic membrane below the plastron. Great care should be taken to limit unnecessary trauma to these structures during a plastron osteotomy and coeliotomy. • If the bladder is accidentally punctured, its contents should be regarded as non-sterile and measures taken to protect the animal from coelomic sepsis. This will probably mean profuse lavage of the pleuroperitoneal/coelomic cavity. • It is unwise to leave an accidentally traumatised bladder without repair as this is likely to result in fatal complications. • It is unclear if trauma to the pericardium requires repair before closure. This author (SM) would advise taking extreme care to reduce associated tissue damage and repair trauma if possible.

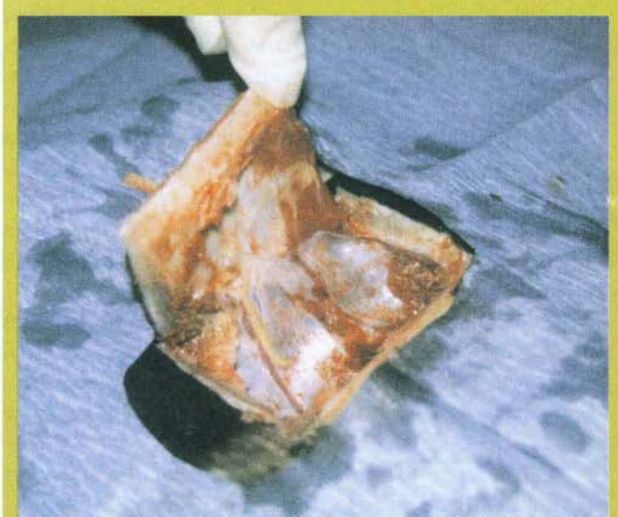

Fig. 15.1 The coelomic membrane is dissected free of the flap. The large, paired coelomic blood vessels are preserved wherever possible (ventral abdominal veins).

Dehydration

In our experience, many chronically ill chelonians are electrolyte and fluid deficient. Ideally, the assessment of each patient should include blood electrolyte analysis. Administration of excessive glucose/dextrose solutions may lead to cerebral or pulmonary oedema. A 50:50 mixture of Ringer's solution and 4% glucose/0.18% sodium chloride is a compromise, which has been widely used with apparent safety in patients for which full blood electrolyte information is not available. Aim for 260–280 mOsm/l.

Jarchow (1988) suggested a rate of 3% of body weight per day in seemingly dehydrated cases until hydration levels are considered normal. 1%–2% of body weight fluids daily or every other day seems well tolerated, in most terrestrial species, for maintenance thereafter. Jarchow (1988) favoured giving chelonians their fluids by the epicoelomic route in situations where the oral route is impractical. This route is also regularly used and encouraged by this author.

Patients that allow stomach tubing without undue resistance and distress, or that have an indwelling oesophagostomy tube, are most safely treated with oral fluids, particularly in the long term. Isotonic intraosseous or intravenous fluids, ideally delivered by syringe driver, are indicated where there is life-threatening acid/base or electrolyte disturbance or severe sepsis.

Hypoperfusion

Where hypoperfusion is suspected (e.g. because of blood loss or sepsis), intravenous or intraosseous administering a colloid or colloid/crystalloid combination, titrated to effect, may be the safest course of action. The administration of crystalloids alone is probably less effective in maintaining circulating blood volume and carries a higher risk of tissue oedema. The synthetic colloid Hetastarch® (Abbot Laboratories, USA), 6% in 0.9% saline, has also been used in chelonians with apparent success (Johnson 2001), however as it is hyperosmotic additional fluid or hypo-osmotic crystalloids may be required to maximise its benefits.

Where blood loss is severe, purified haemoglobin (Oxyglobin®, Biopure) may be required to maintain both circulating volume and oxygen-carrying capacity. Oxyglobin® has been administered via a cardiac catheter to healthy desert tortoises (20 ml/kg given at 99 ml/hr) without apparent adverse effect (Wimsatt 2001: personal communication). Blood transfusions present many potential problems (see Therapeutics for discussion of this issue), however some authors have found them to be beneficial.

Routine intra-operative fluid administration

There are currently no objective data that allow us to monitor whether this practice is beneficial or harmful on an individual case basis. Potential benefits include maintenance of blood pressure, protection of renal function and assisted elimination of drugs and metabolites. Potential adverse effects include pulmonary and cerebral oedema—particularly where renal function is subnormal.

It is very difficult to detect cerebral or pulmonary compromise in the anaesthetised, ventilated patient because our ability to monitor circulation in chelonian patients is limited, however routine use of 8MHz Doppler improves upon this. A flat or pencil probe can be used to detect blood flow in the heart, neck vessels or limb vessels. This allows heart/pulse rate to be detected audibly. The pencil probe must be held in position while the flat probe can be taped over a convenient vessel. Changes in rate/rhythm are easily heard. In addition, the intensity of the signal allows assessment of the 'strength' of heartbeat, i.e. cardiac output. When used on vessels in extremities, it allows assessment of blood flow to these extremities and therefore of tissue perfusion (especially when allied with pulse oximetry). If the animal becomes shocked or hypovolaemic, the strength of signal in the limbs will drop. With experience, this helps determine the efficacy and need for intra-operative fluids.

Temperature and hibernation

Prior to surgery, it is wise to ensure that patients have been maintained within their appropriate temperature range as indicated by measurement of core body temperature using a cloacal probe, as described in the Anaesthesia chapter and by Divers (1999).

Avoid elective surgery immediately prior to hibernation, as anabolic processes will be at their most limited. Inadequate provision of temperature will delay wound healing and compromise the immune response (Bradshaw 1997). It is not yet known how versatile the physiological processes preparing chelonians for hibernation really are. It should be assumed that even after warming a chelonian there will be a time delay or 'rebound time' before a hibernating or imminently hibernating animal can be assumed to have maximised its anabolic capabilities.

Many authors do not allow or advise surgical cases to hibernate the year of their surgery. However in certain cases hibernation may be possible two months after soft tissue surgery or 3–4 months after bone or shell surgery.

PREPARATION OF THE SURGICAL SITE

Table 15.3 describes correct preparation of the surgical site.

SUTURE MATERIALS AND SKIN REPAIR TECHNIQUES

Sutures

Everting suture patterns are recommended for skin closure. Continuous sutures can be used in skin as reptiles seldom self-traumatise surgical sites (Bennett & Mader 1996). Skin suture removal is advised by Bennett (1993a) and Bennett & Mader (1996) regardless of any alleged absorbability of the material used, as skin sutures will persist considerably longer in reptiles than in mammals, and this may lead to complications such as granuloma formation. Table 15.4 discusses individual suture materials.

Glues and patches

Table 15.5 outlines the glues and resins used in chelonian surgery.

WOUND HEALING

Wound healing is well described by Bennett (1993a) and Bennett & Mader (1996). According to these authors, defects fill with proteinaceous fluid and fibrin and this forms a scab. A single layer of

Table 15.3 Preparation of the surgical site.

Positioning	• Position with hot water bottles or heat bags. This author routinely uses five-litre distilled-water bottles supplied for use with autoclaves. Partially filling the bottles and crushing them whilst tightening the lid allows moulding to suit the patient. A moulded bottle will then act as a heated support for the patient during its operation. Zinc oxide or similar tape can be used to anchor the shell at an angle appropriate to the procedure and this allows positioning in almost any orientation. • Towels and sandbags can also be used to stabilise chelonians, especially where background temperatures are relatively high and positioning aids are not also required to heat the patient. Several authors, including Bennett & Mader (1996), advocate a rolled towel in a circular doughnut-like arrangement to cradle the chelonian carapace when dorsal recumbency is required. • Use of a heated bed is illustrated in Figs 15.11–15.12.
Drapes	• Drapes can be see-through and self-adherent (Op-site®, Smith and Nephew) or, double-sided tape can be used to retain cloth or paper drapes.
Disinfection	• The skin and shell are best considered to be faecally contaminated. Creating a sterile field for most chelonian surgical procedures is often difficult. The burrowing behaviour of many chelonians will further add soil, and debilitated animals may sit in their own non-sterile urine. • Adequate cleaning and disinfection of the surgical site are essential. Despite extensive pre-operative patient preparation, a sterile operating field cannot be guaranteed. • Povidone-iodine based disinfectants are suitable to prepare the surgical site. It is suggested that povidone-iodine based products are superior to chlorhexidine in their ability to eliminate pseudomonads (Lloyd & Lamport 1999). • Some authors suggest application of surgical spirit to surgical sites following initial disinfection (Lawton & Stoakes 1992). Although this author does not routinely do so, such application may help to degrease a site prior to application of adhesive drapes.

Table 15.4 The use of suture materials in chelonians.

Monofilament	PDS II® (Ethicon, Johnson and Johnson International) is this author's (SM) suture material of choice in both sterile and contaminated sites.
Metal/nylon haemoclips	Useful during coelomic surgery, such as for ovariectomy.
Skin staples	Of use where skin wounds are of sufficient length and enough free skin is available. The tendency to induce wound eversion is beneficial.
Metal sutures	• Lawton & Stoakes (1992) suggest that metal sutures are useful to stabilise the bony flap of a plastron osteotomy, following coeliotomy and prior to further fixation using epoxy resin or glue (as used by Jordan & Kyzar 1978). • Rosskopf *et al.* (1982) successfully utilised stainless steel sutures during carapacial fracture repair. This is described later. • Metal sutures are not advised in other situations, as monofilament nylon and synthetic absorbable sutures are easier to work with and likely to be less distressing to the patient (Bennett 1998b; Bennett & Mader 1996).
Catgut	The use of catgut should be avoided, as it may provoke chronic inflammatory reactions or harbour infectious agents. Catgut will persist for a considerable time in reptiles, which are believed to lack proteolytic enzymes necessary for its resorption (Lawton & Stoakes 1992; Bennett & Mader 1996).
Braided materials	These may harbour infectious agents more than absorbable monofilament. For this reason this author prefers a synthetic monofilament material where possible.

epithelium migrates below the scab and this then divides to reform a fuller epithelial thickness. Macrophages and heterophils invade the scab and act to protect the wound against infection and clear up any debris or microbial agents present. Table 15.6 describes the factors affecting wound healing.

Skin sutures are generally removed four to six weeks after surgery if wound healing is not compromised by any of the factors described above.

POST-OPERATIVE CARE

Analgesia, appropriate fluid therapy, appropriate nutrition, appropriate heat, light and humidity provision and controlled water exposure are all essential in the immediate post-operative period. Often animals benefit from admission into a therapeutic hospitalisation environment for several weeks, or at least until they are physiologically stable. The reader is referred to the Hospitalisation chapter for further detail.

Some authors advocate elevating patient temperature to the upper region of the ATR during surgical recovery, as metabolism of anaesthetic agents may thereby be increased (Bennett & Mader 1996). It is suggested that a concurrent increase in oxygen consumption will occur, therefore ventilation during recovery should be given to apnoeic patients in order to minimise the risk of hypoxia (Bailey & Pablo 1998).

Temperatures below the ATR will hinder drug metabolism and may prolong anaesthesia. Patient core temperature can be monitored using cloacal or oesophageal temperature probes (Bailey & Pablo 1998). This author uses the probe attachment of the Vet-Ox 4404® (Heska).

Table 15.5 Glues and resins used in chelonian surgery.

Epoxy resins (e.g. Araldite®, Ciba)	Epoxy resins are commonly used with fibreglass sheets to retain bone flaps following reduction in plastron osteotomies (can also be used with gauze swabs). • Epoxy resins such as Araldite® (Ciba) can be used to glue Lego® pieces, wheels and other prosthetic supports onto the chelonian shell.
Epoxy resins and fibreglass (e.g. Fastglas (Isopon)®, W. David and Son)	• This author has had considerable success using fibreglass/plastic sheets and epoxy resin together. The combination is simple and easy to use. Heat production during the setting process does not seem to be a problem as long as the layer is relatively thin, but animals should not be placed on surfaces to which they can stick (e.g. hemp bedding and newspaper) until the resin is dry. After hardening, any still-sticky surface can be covered with Durapore® tape (3M), and the animal can be returned to its normal environment. The tape can easily be removed at any time. The mixture should only be used in well-ventilated areas. • Fibreglass may be used with epoxy resin as described above, and with very good results in shell repair and plastron osteotomy flap replacement (Zeman *et al.* 1967; Rosskopf 1986; Bennett 1998c; Frye 1991a; Mautino & Page 1993; Barten 1996). • Barten (1996) points out that epoxy resins can be tinted with food colourings and textured to mimic the original shell. • Fibreglass is cheap, bonds well to the keratin layer on the outside of the shell and is tough and waterproof. However, it is not a subtle enough material to mould over small areas. • On small areas, Durapore® tape (3M) can be used in single or multiple layers and covered with epoxy resin. A large patch is often required to provide sufficient strength. A fibreglass covering makes visual assessment of the viability of the plastron flap and underlying tissues difficult compared with some of the other techniques.
Shrink-wrap plastic (e.g. Indoor Insulation kit®, Ace Hardware Corp)	Bradley (2000) describes the use of shrink-wrap plastic and waterproof tape (Wet-Pruf®, Kendal Company) to retain dressings over an osteotomy site. A blow drier was used to apply and mould the plastic wrap.
Polymethyl methacrylate (e.g. Technovit®) and similar hoof-repair acrylics	Barten (1996) advises that hoof-repair acrylics are of use in the repair of shell trauma in larger chelonians. Jessop (2000) uses hoof-repair resins and similar materials for routine closure of plastron osteotomies by moulding and applying a gasket-like support to the bony flap. This technique has the advantage of allowing the plastron flap to be visible, which can be helpful where the patient is relatively immobile and may rest on urine or faeces for periods, contaminating the operation site. This technique may be a more appropriate method of closing a plastron osteotomy site in high-humidity species, such as box turtles (*Terrapene* spp.) and red-eared sliders, than fibreglass patches.
Acrylic tissue glues (Superglue®, Loctite Corporation)	• Acrylic tissue glues are recommended by Lawton & Stoakes (1992) for aquatic and semi-aquatic species, where wounds will benefit from being watertight as soon as possible. • Jessop (2000) advises their use to close the skin following microchip implantation. • Cyanoacrylate ester (Superglue®, Loctite Corporation) is also advised by Barten (1996) as an aid to maintaining bandage and dressings in aquatic and semi-aquatic species. This type of acrylic glue can be used temporarily to retain plastic/cellophane coverings over injuries. • This author uses glue to maintain tape on the carapace of water-loving chelonians following oesophagostomy tube placement. A small blob will complement the adhesive of zinc oxide and similar adhesive tapes.
Polytetrafluoroethylene mesh (PTFE) (e.g. Gore-Tex mesh®) and linear polypropylene	A synthetic non-absorbable polytetrafluoroethylene (PTFE) Gore-Tex mesh® was successfully applied to a fistulated and infected plastron osteotomy site of a green sea turtle (*Chelonia mydas*), following the failure and degenerate infection of a previous coeliotomy closure using a fibreglass patch. Linear polypropylene was used as an outer covering to encourage epithelialisation and the site was protected with petroleum jelly and waterproof tape. The turtle was maintained out of water for a month before successfully being reintroduced into a hospital swimming area (Mader 1998d).
Tegaderm™	A clear film dressing of use in open lesions.

Further advice regarding analgesia is available in the Anaesthesia section of this text. The Hospitalisation section of this book gives a guide to suitable post-operative care and facilities.

ADVANCED SURGICAL TECHNOLOGY

Laser surgery

As human medicine and surgery continues to advance, many developments filter through to the veterinary profession, and laser surgery is one such technology. Since Albert Einstein theorized the concept of lasers in 1916, considerable development has produced equipment available to human and, subsequently, veterinary surgeons (Polanyi 1978). The term LASER stands for Light Amplification by the Stimulated Emission of Radiation and relies upon the production of electromagnetic radiation in response to photon emission by the lasing medium (Polanyi 1978; Klause & Roberts 1990).

There is a variety of lasers available, but carbon dioxide (CO_2) and diode models are probably the most common in veterinary

Table 15.6 Wound healing is influenced by a variety of factors.

Environmental temperature	• Inadequate temperature provision compromises both immune response and wound healing. • There is some evidence that maintaining reptiles at the higher end of their appropriate temperature range will maximise healing processes (Smith & Barker 1988). For most chelonians this would generally mean 26°C–32°C. However, account must be taken of increased evaporative water loss if large areas are ulcerated.
Movement and position of the wound	• Wounds on limb extremities will need protection from trauma following surgery. This usually involves bandaging or retention in flexion by adhesive tapes. • Humidity-loving species may experience delayed healing following surgery to the ventral regions of the hind limbs and the prefemoral fossa, as a result of the warm moist environment created around the wound when the hind limbs are maintained in flexion. This effect is increased where patients are immobile and faecal contamination of the area occurs. If this is the case, it is best to avoid provision of heat from below.
Water exposure	• It is often advisable to restrict exposure of aquatic and semi-aquatic species to water in the immediate post-operative period. • Acrylic glues can be used to seal surgical wounds or stick waterproof dressings to the wound site. • Alternatively, waterproof but 'breathable' liquid plastic dressings, such as Germolene New Skin® (SKB), have been used to prevent contamination of open wounds in aquatic mammals (Lucas *et al.* 1999). • Maintenance of water hygiene is of the utmost importance. Species usually resident in water, such as freshwater or marine turtles, benefit from temporary housing in baths or shower units, out of water. • It is common practice for any return to water to be delayed for one to six weeks until healing has taken place, with humidity maintained by spraying and damp coverings. If the animal usually feeds only underwater, it may be necessary to employ nutrition support techniques, such as oesophagostomy tube placement.
Prior infections	• Post-operative infections may occur where debridement of infected lesions is inadequate or impossible. Histology, cytology, culture (aerobic and anaerobic) and sensitivity testing of organisms from affected wounds are usually beneficial. • Where inadequately stabilised patients are presented for surgery the immune system may not be functioning effectively. • Organisms present in other regions of the body may benefit from elevations in temperature and nutritional status before the immune system has the opportunity to rebound and protect. It is therefore good policy to provide systemic antibiotics prior to warming the patient. A broad-spectrum, bactericidal antibiotic should be used. The leucopaenic post-hibernation *Testudo* is a typical example of an immune-comprised patient. Bonner (2000) advises administration of antibiotics before warming such patients, in order to protect against septicaemia.
Environmental hygiene	• Lawton & Stoakes (1992) suggest that paper towels are an ideal post-operative substrate, as regular (daily) replacement will prevent enteric organisms contaminating surgical sites. • Placement of animals into soil or water is not advisable in the immediate post-operative period. • Animals with a preference for high humidity pose greater problems with respect to maintaining environmental hygiene. Dry, regularly-cleaned vivaria suited to a species from arid environments are least likely to harbour faecal-derived contaminating organisms. • Plastic draining-board matting, available at most hardware stores, is helpful in reducing ground contact if animals have plastron damage and prefer a humid environment, especially where animals have limited mobility.
Nutritional status	• Hypocalcaemia, hypoglycaemia and hypoalbuminaemia will adversely affect healing. It is wise to ensure that the previous dietary history of the patient is known and that nutritional support has been given to chronically anorexic or malnourished patients prior to commencement of surgery.

medicine today. The CO_2 laser produces less collateral damage, but the diode laser has the advantage of being compatible with rigid and flexible endoscopes. Both lasers produce an immediate area of vaporisation, surrounded by a zone of irreversible photothermal necrosis and a further zone of reversible oedema. The CO_2 laser can be used either in a focused manner for fine dissection or as a more diffuse beam for tissue coagulation and ablation. The diode laser tip can be coated in a fine layer of carbon, as used in contact mode, with minimal penetration and collateral damage, or used in a non-contact (free beam) mode for tissue coagulation and ablation. All personnel must wear protective eye-wear when using laser devices. The author has successfully used the diode laser in open and endoscopic surgery in a variety of chelonian species for coeliotomy, ovariectomy, salpingectomy/salpingotomy, fibriscess/abscess/neoplasm resection, endoscopic haemostasis and lesion ablation. For a more detailed description of laser equipment and its use in exotic animal surgery, the reader is directed to a recent review by this author (Hernandez-Divers 2002) (Figs 15.2–15.7).

Radiosurgery

Unlike electrocautery (which uses heat), radiosurgery uses high-frequency radio waves of 3.8–4.0 MHz. This technology results in reduced heat production and less collateral damage and is preferred for our more delicate exotic patients (Altman 2000).

The fine, cutting-needle (monopolar) electrodes can be used for incision and dissection, while the bipolar forceps can be

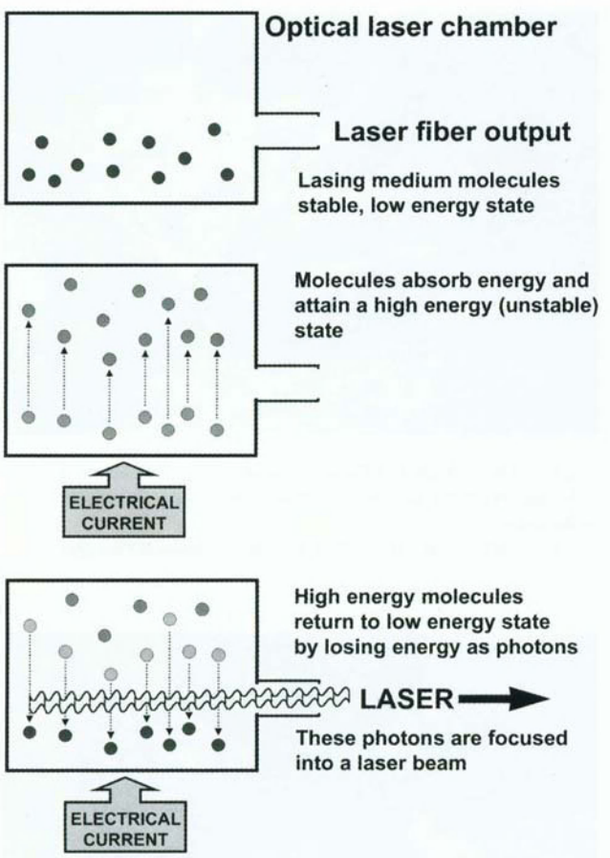

Fig. 15.2 Diagrammatic representation of the production of a laser beam. The top diagram demonstrates the resting lasing medium molecules within the optical laser chamber. As energy in the form of an electrical current is applied, these molecules attain a higher energy level and become unstable (middle). There is a constant effort to return to a more stable state and the molecules achieve this by losing energy in the form of photons (bottom). These photons leave the optical chamber via the laser fibre output as a laser beam.
(Courtesy of Stephen J. Hernandez-Divers, University of Georgia)

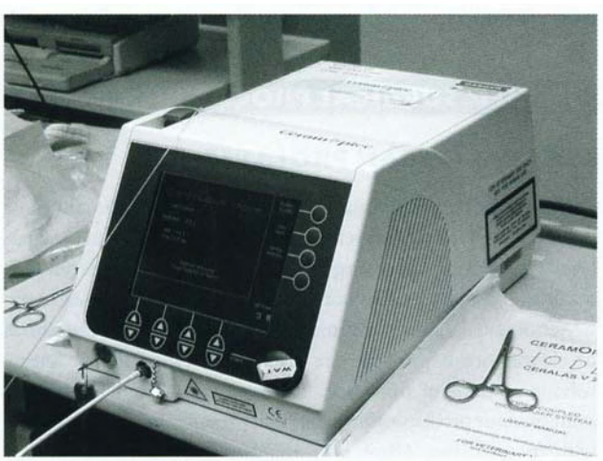

Fig. 15.3 Accuvet Diode laser®.
(Courtesy of Stephen J. Hernandez-Divers, University of Georgia)

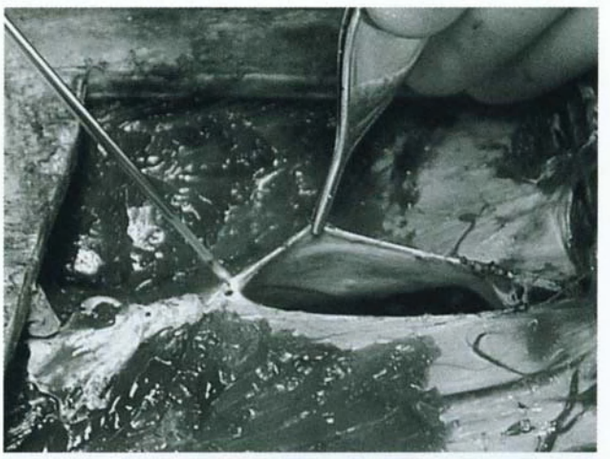

Fig. 15.4 Diode laser incision (contact mode) through the coelomic membrane of an African spurred tortoise (*Geochelone sulcata*).
(Courtesy of Stephen J. Hernandez-Divers, University of Georgia)

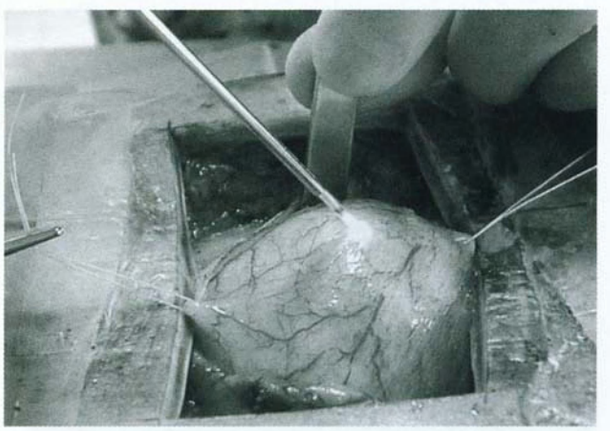

Fig. 15.5 Diode laser cystotomy (contact mode) in an African spurred tortoise (*Geochelone sulcata*).
(Courtesy of Stephen J. Hernandez-Divers, University of Georgia)

Fig. 15.6 Diode laser salpingotomy incision (contact mode) in a leopard tortoise (*Geochelone pardalis*)—note the lack of haemorrhage from the oviduct, and the undamaged egg beneath.
(Courtesy of Stephen J. Hernandez-Divers, University of Georgia)

Fig. 15.7 Using the diode laser (contact mode) to dissect away the oviduct of a Greek tortoise (*Testudo graeca*)—note that the laser seals blood vessels up to 2 mm in diameter making this essentially bloodless surgery.
(Courtesy of Stephen J. Hernandez-Divers, University of Georgia)

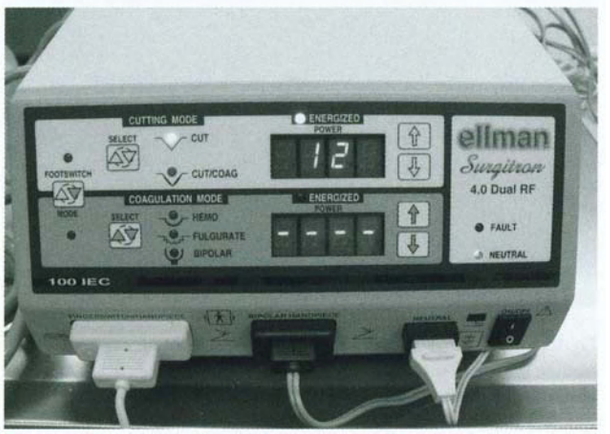

Fig. 15.8 Ellman 4.0 MHz radiosurgery device.
(Courtesy of Stephen J. Hernandez-Divers, University of Georgia)

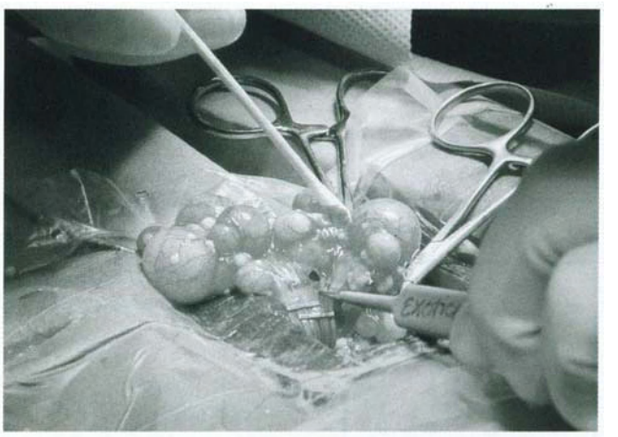

Fig. 15.9 Ovariectomy in a *Terrapene carolina*. Note the use of the radiosurgery bipolar forceps to assist with haemostasis and dissection of the ovary.
(Courtesy of Stephen J. Hernandez-Divers, University of Georgia)

Fig. 15.10 Microsurgery using Surgitel® head-sets (General Scientific Corporation, Michigan). These 4x magnification loupes (one with and one without a focused light source) are light, comfortable and much more versatile than a table-top operating scope.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

invaluable for effecting pinpoint haemostasis of small blood vessels. Radiosurgery devices are also approximately half the cost of a laser and financially attractive to private practitioners. Ellman International Inc. (Hewlett, NY) manufactures a 4.0 MHz machine (the successor to the older 3.8 MHz) which the author has used for open and endoscopic surgery in many reptile species. The newer machine can have both monopolar and bipolar devices plugged in simultaneously, with foot-pedal activation of bipolar electrodes and finger-switch activation for monopolar electrodes (Figs 15.8–15.9).

Additional surgical equipment

Further advanced surgical equipment of use in the management of chelonians is illustrated in Figs 15.10–15.12.

SPECIFIC SURGICAL PROCEDURES

CLOACAL ORGAN PROLAPSE

It is important to differentiate normal protrusion of the clitoris or penis through the cloacal vent from a pathological organ prolapse (Fig. 11.97). Mild, non-surgical, penile or clitoral prolapse may occur as a result of a variety of simple conditions described later.

Aetiology, diagnosis and management of predisposing causes of cloacal organ prolapse are also described in the problem-solving sections of this book. It is essential that the patient is examined for predispositions and that these are treated in tandem with the prolapse. Administer analgesics, fluids and antibiotic cover to all patients with a prolapse once systemic evaluation has been performed.

Predisposing causes include general debility, neurological dysfunction, any coelomic space-occupying lesion, any cause of straining (dyspnoea, constipation, egg retention, oviposition,

Fig. 15.11 Bair Hugger® warming unit Model 500 (Augustine Medical, Inc.,10393 West 70th Street, Eden Prairie, MN 55344).
The base unit warms air to a set (adjustable) temperature.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

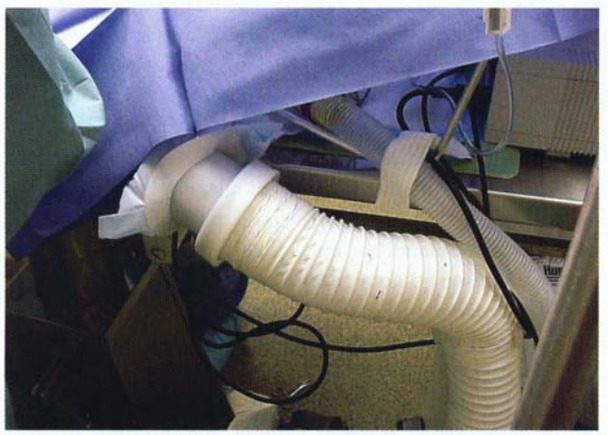

Fig. 15.12 Bair Hugger® warming unit Model 500 (Augustine Medical, Inc., 10393 West 70th Street, Eden Prairie, MN 55344).
Warmed air is circulated through a fenestrated table mat bathing the patient in warm air.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

cystic calculi), metabolic disease (dehydration, hypocalcaemia, ketoacidosis, hyperoestrogenism), cloacal hypertrophy, obesity, excessive libido, bacterial, fungal, viral and parasitic infections of the lower genitourinary or digestive tracts (Figs 11.97–11.103). Penile prolapse (paraphimosis) may also be the result of bites from cage-mates (where semi-aquatic species are maintained in an overcrowded aquarium), contamination of the penis with bedding material and trauma during copulation (Zwart 1992).

Table 15.7 Identifying the prolapsed organ.

Sex of animal	Description of prolapse	Likely organ
Female	Large and sac like, fluid filled	Bladder
	Tubular—intussuscepted	Large intestine
	Tubular—intussuscepted	Oviduct
	Large and sac like, solid tissue	Cloacal tissues
Male	Large and sac like, fluid filled	Bladder
	Pedunculated—linear	Penis
	Tubular—intussuscepted	Large intestine
	Large and sac like, solid tissue	Cloacal tissues

Identification of the prolapsed structure

Various structures may become prolapsed through the cloacal opening. These include rectum, oviduct, penis, bladder and the cloaca itself. Efforts should be made to try to identify exactly what it is that has prolapsed. Prolapse of ureters has not been described.

It is important to know whether or not urination and defecation are continuing around the prolapse. The sex of the animal may be significant, and the morphology of the prolapse (intussuscepted, tubular, pedunculated, etc.) may give important clues (Figs 11.100–11.103). Table 15.7 may help to identify the prolapsed organ.

Analgesia

Local or general anaesthetic agents and post-operative analgesics are discussed elsewhere and should be administered where surgery is necessary. They may also benefit any animal with a prolapse, especially if it has become traumatised.

Prolapse reduction

Delays in reduction may result in trauma to the organ, venous congestion, strangulation and ischaemic necrosis. Attempts should be made to lubricate and reduce a prolapse as soon as possible (Fig. 13.2). Owners and keepers should be encouraged to rinse any prolapse under running water before wrapping the caudal body in plastic food wrapping. This helps reduce further tissue damage and desiccation during transportation. Problems revealed during the evaluation process, such as dehydration, sepsis and hypocalcaemia, require treatment at the same time. After appropriate stabilising measures have been taken, a recent and potentially viable prolapse should be cleaned and either reduced or protected.

If early reduction is not possible, application of lubricants, such as petroleum jelly, water-soluble lubricating jelly, antibiotic ointment, or a moist dressing of some description will help protect the prolapse (Rosskopf *et al.* 1982 suggested damp towels).

If a prolapsed penis is swollen, Boyer (1992b) suggests that soaking in 50% dextrose may help reduce tissue oedema.

Alternatively, the oedematous organ can be wrapped in paper towels soaked with 50% dextrose. The towel bandage inhibits new fluids from accumulating and the dextrose aids draining of the prolapsed tissue.

Blunt instruments, digits, rubber stomach tubes and gentle water pressure can all be used to invaginate or invert structures, such as oviduct, bladder or rectum, which have become intussuscepted (Figs 13.3–13.5). The bladder can be drained of fluid prior to reduction by cystocentesis.

It is best to try to conserve the prolapsed material wherever possible when treating bladder and rectal/large intestinal prolapses.

Episiotomy

Where, despite osmotic reduction an engorged organ is too swollen to replace, a linear releasing incision (episiotomy) to the margin of the cloaca may ease replacement. This should be sutured post-operatively using fine subcutaneous sutures of PDSII® (Ethicon).

Purse-string sutures

Indications

DeNardo (1996) suggests that when an oviduct has been adequately inverted, reduced and replaced, a retentive purse-string suture is not generally necessary. The opposite is often the case with penile prolapses. The use of purse-string sutures is not guaranteed to resolve a prolapse. Following release, the organ involved is often found simply to have been crammed into the cloaca in a similar or worse state than prior to its reduction. This author (SM) advises cautious use of purse-string sutures, and suggests that clients should be informed that further surgery, including organ amputation, might still be required.

Placement of any purse-string suture should take into account the animal's need to urinate or defecate, and a clinician should be aware that an inadequately-reduced necrotic organ may be provided with an environment suited to serious secondary bacterial infection if bathed in faecal material and incubated within the animal's ATR.

Technique

The technique is illustrated in the accompanying plates (Figs 13.6–13.7). Boyer (1992b) and many other American authors such as Frye (1992) and Bennett (1995) advise that purse-string sutures to retain a penile prolapse should be left in place for around three weeks. Barten (1996) suggests two weeks and Jackson (1991) and Lawton & Stoakes (1992) suggest that five to ten days may suffice. During this period, other problems should be addressed.

Prolapse amputation

A chronically-prolapsed structure that has become necrotic or heavily infected is often best removed.

The penis is the most commonly-removed structure (Figs 13.8–13.9). Partial cystectomy or enterectomy are possible at coeliotomy through a plastron osteotomy, but potentially serious complications are likely to ensue. In comparison, oviductal or penile resection is generally successful (Figs 13.10–13.15). Cloacal resection has also been well tolerated in animals treated by this author (SM) (Figs 13.16–13.18). Successful removal of a prolapsed inflammatory cloacal lesion, using electrosurgery, was also described in an adult male Galapagos tortoise (*Geochelone nigra*) (Ensley & Lanner 1981).

Penile amputation

Indications

When a penile prolapse has been seriously traumatised, amputation may be the most sensible treatment option. Following penile amputation, a tortoise will be infertile, but urination and behaviour are normally unaffected. The penile urethra is not closed and it plays no significant role in urination. The penis arises from the floor of the cloaca.

Technique

The technique employed by the author (SM) is illustrated in Figs 13.8–13.9. Post-operative care is generally minimal. Antibiotic ointment is often placed into the cloaca. Systemic antibiotics may also be indicated. Efforts should be made to investigate and manage any concurrent problems that may have predisposed to the prolapse in the first place. Follow-up checks should be made to observe for possible post-operative infection. Cloacoscopic evaluations are recommended.

Amputation of prolapsed oviductal material

Indications

Oviductal prolapse can occur during normal oviposition. Application of traction and drugs such as oxytocin may be predisposing factors (DeNardo 1996). Oviductal prolapse at times other than oviposition or gravidity may relate to metabolic imbalances, such as hypocalcaemia. Oviductal prolapse is reported as relatively uncommon in the literature but is regularly presented in practice.

In some circumstances a prolapse can be reduced and a purse-string suture applied as described earlier. However it is not easy to guarantee that uterine material has adequately involuted and been returned to its normal coelomic position. Similarly, the effect of the displacement upon the ovaries and their follicles will be unknown without coelomic inspection. Where the prolapse has become avascular, infected or traumatised and where coelomic endoscopy or other physical signs suggest compromise of ovarian tissue, then coeliotomy, ovariectomy and amputation of the prolapse are indicated. It is not advisable to amputate traumatised uterine material without consideration of what is happening within the coelom. Where possible, cloacal and coelomic surgery should be combined.

Bennett (1993b) describes plastron osteotomy, coeliotomy and ovariosalpingectomy following a uterine prolapse in a desert tortoise (*Gopherus agassizii*). In this case the prolapse appeared to be secondary to a large cystic calculus. The bladder stone was also removed through a simultaneous cystotomy.

Nutter *et al.* (2000) describe successful hemisalpingectomy in a loggerhead sea turtle. The turtle was found with a 1.5 m traumatised cloacal prolapse, identified as oviduct. A curvilinear, soft-tissue, flank approach was made in the prefemoral fossa and

oviductal remnants and associated right ovary were removed. Following rehabilitation and release, the turtle was observed nesting two years later. She produced multiple clutches of eggs, of greater than normal clutch size, but with normal hatch rate.

Technique

Amputation with and without ovariohysterectomy is described by Frye (1974), and the procedure is illustrated in Figs 13.10–13.15.

Amputation of prolapsed cloaca/rectum

Indications

Prolapse of the cloaca and rectum without a simultaneous coelomic mass, such as a urolith, or dystocia/gravidity is unusual. Early reduction and retention using a purse-string suture is desirable, but amputation is occasionally necessary with potentially favourable outcome even in severely-traumatised cases.

Technique

Removal of portions of the large intestine involves coeliotomy and anastomosis of the resected intestine. This is complicated, as exposure is limited through a coeliotomy site. Alternatively blind removal of prolapsed material using circumferential mattress sutures can be considered. Both these procedures are ambitious and prone to complications.

EAR ABSCESSES

Tympanic infection is a common presentation in all chelonians. Vestibular signs are not usually present with abscessation or following surgery. Most ear abscesses present as swelling and enlargement of the tympanic scute. This is found on the lateral aspect of the head. Solid caseous material usually fills the middle ear and extends down the Eustachian tube. Intra-oral examination of both Eustachian tube openings within the pharynx is advised. The condition is often bilateral and unequal (Figs 13.20–13.22).

Ear abscesses appear to be the result of infection extending up the Eustachian tube (Jackson 1991), and may reflect poor environmental hygiene and oro-faecal contamination. This is especially true of semi-aquatic and aquatic animals kept in poorly-maintained water. Cytology may quickly confirm if infecting organisms are bacterial or mycotic. Sample collection for culture and sensitivity testing prior to administration of any antimicrobial is sensible.

Immunosuppression, e.g. inadequate temperature provision, poor water quality, chemical exposure (Tangredi & Evans 1997) or nutritional diseases (e.g. hypovitaminosis A) may be predisposing factors. Haematogenous and traumatic origins are also plausible (Murray 1996). A full work-up, paying particular attention to captive environment and nutrition, is indicated. Concurrent disease is likely. Concurrent disease and dispositions require appropriate investigation and management in addition to any specific management geared to resolve the ear abscess.

Infection may spread locally resulting in osteomyelitis of the jaw and skull. Survey radiographs of the skull are important to evaluate the extent of the pathology and to plan the amount of surgical debridement necessary. Occasionally, the skin around the tympanic scute will be affected by a local cellulitis, which can spread as far as the orbit. Left untreated, an ear infection may well disseminate and predispose to, or present as, a septicaemia.

Indications

Treatment of a chelonian ear abscess is usually surgical, as most tympanic abscesses encapsulate and generally contain inspissated pus, which is not readily penetrated by parenteral antibiotics.

Technique

During stabilisation, antibiotics and analgesics should be considered. Pre-operative antibiotics are best given following collection of a sample for culture and sensitivity which can be used to modify antibiotic protocol if sensitivity testing shows the infection to be resistant to the chosen antibiotic. The procedure is outlined in Table 15.8 and Figs 13.23–13.32.

Table 15.8 Surgical treatment of ear abscesses.

(1) Once stabilised the animal is best anaesthetised and intubated. This author does not advise restraining and operating on such infections without adequate analgesia, a procedure occasionally advocated by ambitious and misguided keepers.

(2) Often drapes are a hindrance, but both eyes should always be protected during preparation of the surgical site, especially if spirit or other irritant preparations are used. Care should be taken to ensure the eye isn't damaged beneath drapes or during attachment of towel clips to the patient.

(3) Curettage of an ear abscess is a relatively easy surgical procedure broadly equivalent to a lateral bulla osteotomy in a mammal. Volkmann's curette or another similar instrument may help debride any local invasion of the tympanic capsule or surrounding bone.

(4) Haemorrhage is generally limited. Any continued bleeding will normally cease with digital pressure or light cautery/electrosurgery/radiosurgery.

(5) The Eustachian tubes should be assessed and flushed after the middle ear has been cleaned surgically. Flushing will generally assist in the removal of most debris. A small irrigating cannula is easily passed into the Eustachian tube. This is then flushed with saline with the animal intubated. A swab placed in the pharynx will help remove excess and contaminated fluid and reduce possible ingestion or aspiration. Patency of the tube is important to reduce the likelihood of recurrence.

(6) The soft tissues and scute overlying the ear are not usually closed following this type of surgical procedure, and antibiotic cover and analgesia are generally provided until the site has healed adequately and pain has resolved (Fig. 13.30).

(7) Poor environmental hygiene and the various potential causes of immunocompromise such as poor temperature provision, inadequate nutrition and even exposure to immunosuppressive chemicals (Tangredi & Evans 1997) should be investigated and addressed as appropriate.

(8) Other treatment options beyond administration of mere systemic antibiosis include the local use of implanted antibiotic impregnated beads and cryosurgery (care must be taken to protect local nervous tissue and the eyes where cryosurgery is employed).

Fig. 15.13 This hard mass on the dorsal aspect of a hind limb is typical of an injection site abscess. Often animals with these will have been given a post-hibernation vitamin injection at some point in the recent past.

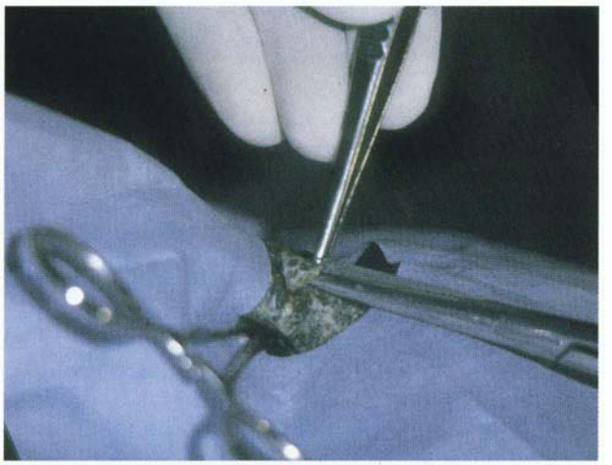

Fig. 15.14 After appropriate skin preparation, the lesion can be draped and removed. It is advisable to send material for culture and sensitivity and to consider the possible need for mycobacterial culture. The sample can be divided into three and dealt with as discussed in the text.

SUBCUTANEOUS ABSCESS/FIBRISCESSES

Chelonian abscess/fibriscesses generally contain solid, caseous material and therefore differ from the typical liquid abscesses of most mammals. Abscess/fibriscesses are found in various sites. The ear, limbs, injection sites, around the neck and retrobulbar sites all appear common. Abscess/fibriscesses around the neck often result from self-inoculation of foreign material such as bedding substrate (e.g. bark) during head retraction. Abscess/fibriscesses of the limbs are occasionally the product of poor aseptic technique or bad luck when injecting medications. Mycotic, bacterial and mycobacterial infections of the skin are all reported (Figs 15.13–15.18).

Technique

Surgery is generally performed under general anaesthesia, as local anaesthesia is likely to affect wound healing (Table 15.9).

Fig. 15.15 Many abscesses are not discrete, circumscribed or encapsulated. Such infections may be best treated with medications according to isolate sensitivity patterns.

The lesion on this red-eared slider was preventing head retraction and failed to respond to several weeks of antibiotic therapy prior to referral.

Fig. 15.16 Surgical removal of all visibly abnormal tissue, including a margin of safety, is indicated where a lesion is causing pain or has not resolved with medical treatment.

COELIOTOMY

The visceral organs of the chelonian all lie within the coelomic cavity. The coelomic cavity is generally encased in a bony vault created by the carapace and plastron. In order to reach the coelomic cavity the surgeon is forced either to cross the dermal plates of the carapace or plastron, or to take a soft-tissue approach through the prefemoral fossa. A prefemoral, soft-tissue approach appears relatively straightforward as little hardware is required, but access and procedures possible are limited. A transplastron approach requires equipment to open the plastron and material to close it. A visual assessment of all available coelomic organs is prudent whenever a coeliotomy is performed.

Fig. 15.17 A sliding 'T' advancement flap has been used to cover the defect created by surgical excision of skin at a site where there is little free skin.

The skin is everted using PDS II® (Ethicon, Johnson and Johnson International) mattress sutures and healed without complication. Tension on the surgical site was not a problem and this slider's neck was easily extended and flexed following surgery.

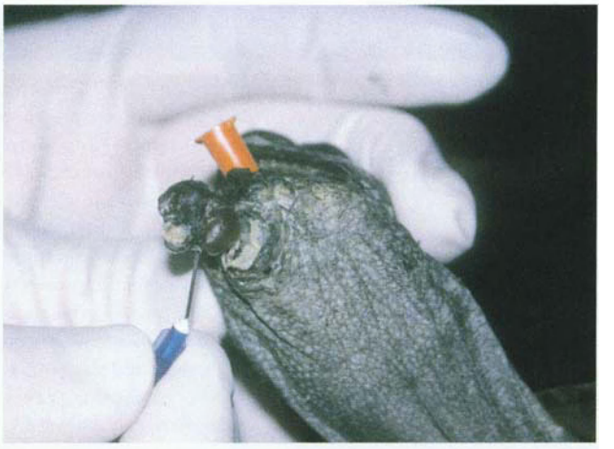

Fig. 15.18 Lesions such as this may contain Mycobacteria. In this case only a fungal agent was identified.

The lesion in Fig. 15.17 shelled out easily and the site resolved without complication, with daily post-operative cleaning with a dilute povidone-iodine solution. The client declined more aggressive treatment and a satisfactory recovery was made over the following two years.

Table 15.9 Surgical treatment of abscess/fibriscesses.

(1)	The surgical area should be aseptically prepared and neighbouring areas draped in order to reduce possible cross contamination.
(2)	If there is a capsule and it and its contents can be totally excised, then the skin over the abscess/fibriscess can sometimes be closed. Circumscribed, discrete abscess/fibriscesses where there is plenty of skin are easiest to excise completely with tissues sutured to produce first intention healing. Reptile abscess/fibriscesses are occasionally more disseminate than those of mammals, and capsules may be poorly defined, making a primary closure technique prone to potential recurrence.
(3)	It is wise for a sample or swab taken at the start of treatment to be dispatched for culture and sensitivity testing. (Check for fungi, mycobacterium, anaerobes, and aerobes.) Animal-side cytology may quickly determine whether bacteria, mycotic agents or mixed infections are present.
(4)	Curettes used for other forms of surgery may be helpful during debridement.
(5)	Attempt to remove all visibly infected tissue and consider taking a further margin where possible without compromising the animal.
(6)	Often mobilisation of free skin to fill in defects is complicated by its attachments to the carapace or plastron. There is a lack of free skin around the head and appendages. Here it may be more practical to leave the surgical site open to heal by second intention. Advancement and rotational flaps are occasionally effective.
(7)	Where *Mycobacterium* infection is suspected, it is wise to divide any material removed into three portions: the first can be dispatched for culture and sensitivity; the second can be dispatched for histopathology; the third can be moistened and retained in a refrigerated state and sent for appropriate and potentially prolonged mycobacterial culture if acid-fast organisms are apparent on histopathology.
(8)	Solid abscess/fibriscesses often shell out during physical examination and often do surprisingly well if just left open and well ventilated to heal.
(9)	Where complete excision of an encapsulated lesion is not possible, the surgical area should be appropriately flushed with a diluted disinfectant, such as chlorhexidine or povidone-iodine, whilst protecting surrounding structures from contamination.
(10)	Where particularly resistant organisms with no appropriate antibiotic treatment are encountered, or recurrence and cellulitis have occurred, cryosurgery of the affected area with post-operative exposure/ ventilation may prove to be of value.
(11)	Post-operative flushing and lesion management should be monitored over the following few weeks. Keepers should be encouraged to return animals for regular post-operative checks.
(12)	Antibiotics and antifungal agents are best administered in response to sensitivity patterns determined by culture of the offending isolate. A broad-spectrum antibiotic can be administered at the time of surgery and this can be altered upon the return of the culture and sensitivity result if the organism present proved to be resistant. Agents able to deal with anaerobes may be advisable. Further comment on antibiotic preparations and combinations is given in the Therapeutics chapter.

Choice of approach

Size and preferred environment are factors affecting choice of approach:

- Small terrestrial chelonians less than 5 kg from medium- or low-humidity environments are generally good candidates for a central plastron osteotomy.
- Larger terrestrial species are good candidates for a soft-tissue flank approach through the tissues of the prefemoral fossa.
- Medium to large aquatic and semi-aquatic species are good candidates for a soft-tissue flank approach through the tissues of the prefemoral fossa.
- Small aquatic and semi-aquatic species may require a combined soft-tissue and lateral plastron osteotomy approach or a central plastron osteotomy with appropriate management of wound and water exposure post operatively.
- Burrowing, high humidity species are good candidates for either a prefemoral or a plastron approach.

Table 15.10 Suitability of differing approaches for different chelonians.

Central plastron osteotomy	Entry through ossified dermal plates requires some form of mechanical saw or burr and results in considerable trauma. Coelomic access is generally restricted to the central portion of the plastron (the femoral and abdominal shields). Analgesia and a post-operative method of replacing the plates and repairing the defect are required. From personal experience (SM) it would seem that plastron osteotomy complications increase with post-operative humidity and water exposure. As water exposure/bathing must be restricted in the immediate post-operative period, high humidity and aquatic species do not tolerate temporary plastron osteotomy as well as do terrestrial chelonians from arid environments, unless post-operative conditions are exceptionally well controlled. • Underlying the carapace are the lungs, vertebra, and spinal cord. These preclude the use of this surface for a surgical approach to the coelom. • The cranial plastron is attached to the pectoral girdle and associated musculature and the pericardial sac, whilst the caudal plastron is attached to the pelvic girdle and its associated musculature. • Anterior to the pelvic girdle is the bladder, which can vary considerably in size and may complicate surgical access when fully distended. • This really leaves the central plastron as the most useful entry point, although available space will vary upon bladder distension at time of surgery.
Soft-tissue flank approach through the prefemoral fossa	In species where the plastron is relatively small, entry to the coelomic cavity through the soft tissues of the prefemoral fossa is possible. Morbidity and healing time are decreased. • Access is severely restricted in small individuals compared to large. • Surgery is less challenging as chelonian size increases. This approach is described in various species such as soft-shell turtles (*Trionyx* spp.) and snapping turtles (*Chelydra* spp.) (Lawton & Stoakes (1992), and the desert tortoise (*Xerobates* spp.) and Olive Ridley *Lepidochelys olivacea* (Isenbugel & Barandun 1981).
Lateral plastronotomy combined with prefemoral approach	In semi-aquatic and aquatic specimens it is possible to create a small hinge in the lateral plastron in order to increase exposure. This approach may have benefits over a more central plastron osteotomy, as the animal may become waterproof sooner post operatively, and is therefore able to tolerate water exposure earlier.

Table 15.10 summarises the suitability of different approaches depending upon the patient.

CENTRAL PLASTRON OSTEOTOMY

Chelonian coelomic surgery presents special problems because of the constraints imposed by the protective shell. In the majority of terrestrial species there is inadequate soft tissue access to the coelomic cavity and an entry to the coelom through the plastron cannot be avoided. In larger animals and especially in marine species the plastron is relatively small in comparison to the carapace and it becomes more realistic to attempt a soft-tissue coelomic approach via the prefemoral fossa.

Oscillating saws, dental drills, orthopaedic air drills and modelling burrs have all been used successfully to cut through the chelonian plastron in order to achieve a surgical entry and approach to the coelom. The technique and equipment used are described below, however there are a variety of equally useful alternative methods and any descriptions given here are not intended to imply that alternatives will not be equally as effective.

Indications

Some indications for plastron osteotomy and coeliotomy are:

- exploratory surgery;
- organ biopsy;
- management of coelomitis;
- gastrotomy/enterotomy for removal of intestinal foreign body/obstruction/intussusception/resection of the intestinal tract;
- urolith removal;
- ovariectomy;
- salpingotomy and removal of pathologically retained ova;
- reduction and/or resection of cloacal prolapse.

Preparation

The technique commonly employed is illustrated and the reader is referred to the accompanying plates (Figs 15.1, 15.19–15.56).

Many important pre-operative considerations must be addressed before embarking upon surgery. These considerations include: surgical suitability (age/concurrent disease), pre-operative stabilisation through hospitalisation, anaesthesia/analgesia/ventilation, pre-operative antibiosis, fluid therapy before during and after surgery and environmental temperatures before during and after surgery. These are also discussed in the Anaesthesia and Hospitalisation sections.

The bladder and pericardium are large membranous structures that must be identified and protected during coelomic entry, surgery and exit. It may be advisable to operate immediately after spontaneous urination, or to stimulate urination or to drain bladder fluid through catheterisation as described elsewhere in this book under fluid therapy.

Table 15.11 summarises preparation for plastron osteotomy.

Plastron osteotomy

Some authors suggest that any burr or cutting blade should be cooled with liquids in order to reduce heat necrosis. In this author's (SM) experience heat necrosis has not been a problem using the equipment described. Excessive wetting merely throws

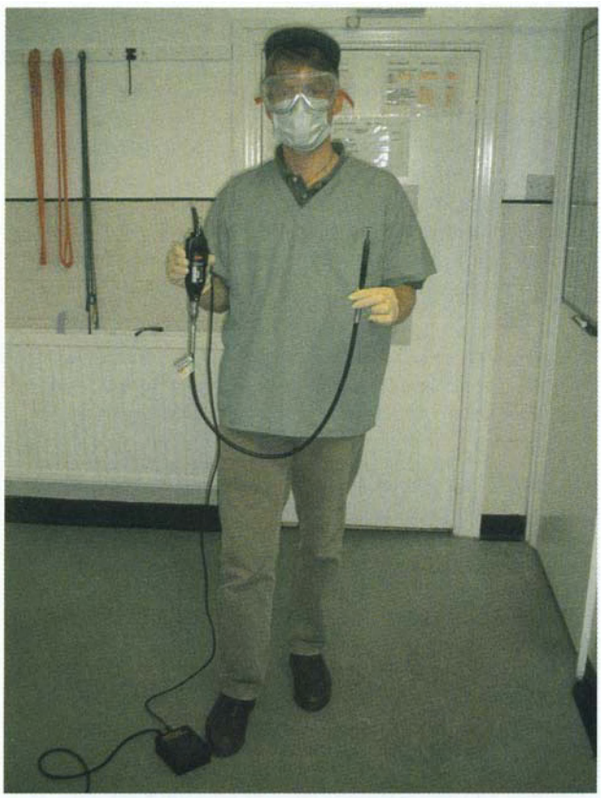

Fig. 15.19 Equipment used by the author for coeliotomy includes a burr or cutting disc attached to a high-speed drill with a flexible extension and footswitch (and 1 m flexible extension: Dremmel®, Footswitch—221-Type 2: Dremmel®, Diamond dental cutting blade–125® Intensive Swiss—or a long-handled dental root burr). Some authors suggest spay hooks to be helpful in exteriorising viscera.

Fig. 15.20 The Dremmel® Multi 10 000–37 000 is an ideal tool for use during plastron osteotomy or post-mortem examination.

Fig. 15.21 3M mini driver® and hand saw attachment (MicroAire Surgical Instruments Charlottesville, VA).
Minimal oscillations limit soft-tissue trauma.
(Courtesy of Stephen Hernandez-Divers, University of Georgia)

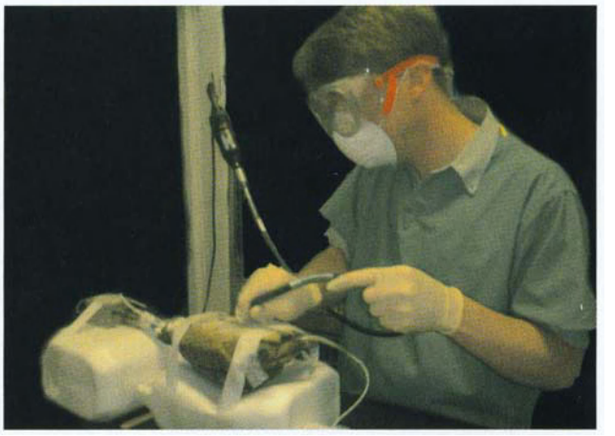

Fig. 15.22 Ideally the operator should wear protective eye goggles and a facemask, as a lot of airborne debris is created during the burring process.

fluid and debris about the room, and at the surgeon, although drapes can be used as shielding where water is employed. A great deal of debris will be created (Fig. 15.29).

The flap can be cut on all four sides and removed, or it can be cut on just three sides and reflected back on the fourth through a scored hinge. This author favours the latter technique. Reflecting a flap, and maintaining soft tissue attachments where possible, may increase the viability of the flap, and therefore speed healing, although healing relates more closely to atraumatic handling and repair of the coelomic membrane, which acts as a centre of ossification and forms new bone post operatively.

When the osteotomy flap is removed, it should be protected. Immersion in sterile saline and exposure to antibiotic powders are contraindicated, as they appear to inhibit osteogenesis (Gray & Elves 1979; Gray & Elves 1981). It may be best to wrap the

Table 15.11 Plastron osteotomy patient and operation preparation.

Equipment and materials	This author (SM) generally opens the shell using a burr or cutting disc attached to a high-speed drill, with a flexible extension and footswitch (Dremmel® Multi 10 000–37 000 and 1 m flexible extension; Dremmel® Footswitch—221-Type 2; Dremmel® Diamond dental cutting blade—125® Intensive Swiss—or a long-handled dental root burr) (Figs 15.19–15.21). Other authors have used air drills with bone saw attachments with success. Some authors suggest that spay hooks are helpful in exteriorising viscera, although this author does not use them. For materials suitable for plastron repair, see Table 15.5. Orthopaedic fixation implants and wires may also be used.
Patient preparation and protection	The patient is likely to be draped during the procedure. Clear drapes help the surgeon to remember that structures such as the limbs are more delicate than the shell, and may become traumatised if excessive force is used, or if the surgeon leans on the wrong place. The surgery will take some time, and the surgeon's mind is likely to be focussed on matters other than the limbs. It is therefore important that the appendages are protected, so that leaning and leverage cannot cause ligament damage and fracture. Strapping the limbs in flexion, using simple tape such as Durapore® (3M), can help avoid inadvertent trauma (Fig. 15.23).
Ventilation	The patient generally requires ventilation. Placing the patient on an inclined support, with the cranial carapace inclined at an angle of approximately 20°, ensures that the weight of the viscera does not prevent the cranial lungs from inflating (Fig. 15.25). The caudal lung fields suffer greatest mechanical compromise during dorsal recumbency associated with surgery. Ventilation is described in the Anaesthesia section of this book.
Patient positioning	The patient generally requires dorsal recumbency throughout the surgery. Various positioning aids have already been described. This author (SM) tends to tape the animal to five-litre plastic water bottles. The bottles are filled with warm water at the start of surgery, and can be crushed to suit the contours of the patient's carapace, the lid being tightened when the bottle reaches the desired shape (Fig. 15.24). If this is tricky, it can be more easily performed with the bottle submerged in warm water. Sand bags and sponge are also suitable. The ventilator can be placed on a similar dimension support immediately in front of the patient.
Marking surgical site	The size and location of the entry flap should take into account underlying structures such as the bladder, pelvic musculature, pectoral musculature and heart, which should all be avoided unless the surgery specifically requires access to them. Marking the entry site prior to burring is useful (Fig. 15.25). • Pre-operative radiographs help to locate (and so avoid) important structures, and can be used to help formulate a surgical plan. • The osteotomy size is determined by the size of eggs, uroliths or foreign bodies that are to be removed. It should be large enough not only to pass these objects through, but also any device (forceps, spoon, lever, etc.) holding them.
Score the osteotomy outline	After marking the proposed flap site with marker pen, and gently scoring with the burr, the site can be prepared without the risk of washing off markings (Fig. 15.26).
Surgical preparation	Strict asepsis is essential. The plastron must be considered to be faecally contaminated. It also has many crevices and fissures. • Toothbrushes and scrub brushes can access and clean sites that cannot be reached with swabs or cotton wool (Fig. 15.24). • It is important to monitor core body temperature during this procedure. Bear in mind that the application of excessive/cold fluids may chill the patient through evaporative heat loss. Excess fluid should be wiped away using sterile towelling. • Drape the animal twice—before and after the cutting of the plastron. An alternative approach is to apply two drapes and to remove the outer drape and associated debris after the osteotomy has been cut, leaving the clean under-drape as the surgical protection. • The osteotomy site needs aseptic preparation before the flap is cut. • A repeat clean will then be required, and care must be taken to prevent debris entering the coeliotomy site (Fig. 15.27).
Operator protection	The surgical and nursing/anaesthesia staff should all wear protective eyewear and facemasks during the incision of the plastron (Fig. 15.22). Epoxy resins are intoxicating solvents and should be mixed in well-ventilated areas. Protective wear can be discarded later when airborne plastron particles are no longer a threat. The room should be well ventilated with adequate air extraction and turnover.

plastron section in moistened, blood-soaked sponges, which are again wrapped in saline-soaked sponges or damp swabs, until replacement.

Cutting through the plastron at an angle makes the inner aspect of the flap smaller than the outer. This ensures that the flap will not fall into the tortoise when replaced, and can be easily fixed back in position (Figs 15.28, 15.35–15.36).

Once three sides have been cut full thickness, the fourth side can be cut to half the thickness, and a wedge of bone burred away. This allows this edge to be reflected as a hinge. The side to which

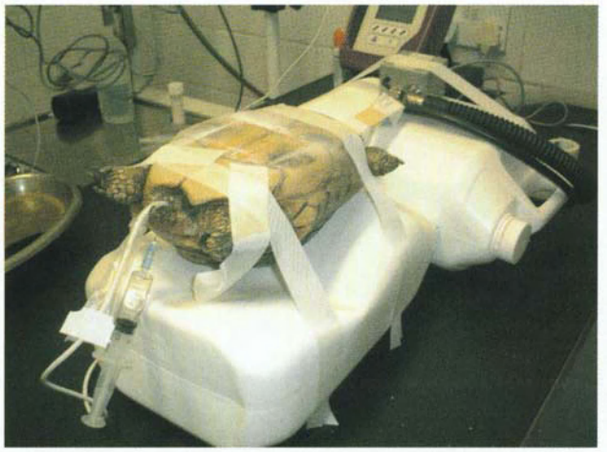

Fig. 15.23 Limbs are protected from inadvertent trauma by taping them in flexion throughout preparation.

Historically, this author (SM) has used incremental Saffan® through a syringe left conveniently placed in the dorsal coccygeal vein. This has given effective anaesthetic maintenance without complication in over a hundred cases.

Jugular injection and maintenance with volatile agents via ventilation are alterative options.

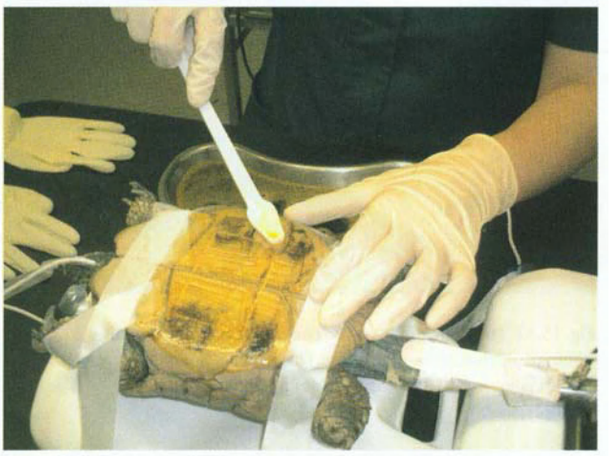

Fig. 15.24 First-stage preparation involves thorough cleaning and disinfection of the plastron, which may be heavily contaminated with faecal organisms. Here a small brush is used.

The hinge is reflected depends upon the structures the surgeon is hoping to reach. Usually the caudal incision acts as a hinge, as this affords better access to coelomic structures such as the bladder, oviducts, uterine horns and liver.

A sterile orthopaedic screwdriver or periosteal elevator can be used to open and lever up the bone flap. The flap is reflected gently and muscular and soft-tissue attachments dissected away on three sides, but maintained on the fourth if the flap is to remain attached (Figs 15.30–15.31, 15.1).

Entering the coelom

The coelomic membrane is dissected free of the flap. Where possible the large, paired, ventral abdominal blood vessels, running parallel to the midline in a craniocaudal direction, are preserved.

Fig. 15.25 Placing the patient on an inclined support so the cranial carapace is inclined at an angle of approximately 20° ensures that the cranial lung fields are easily ventilated without excessive compression from the weight of overlying viscera. The caudal lung fields may not be ventilated so easily but are sacrificed in favour of the cranial fields.

Fig. 15.26 The plastron osteotomy outline can be gently scored using a burr. Therefore it is not important that pen markings may be washed off during surgical preparation of the plastron.

Fig. 15.27 The coeliotomy site has been marked on the plastron and the plastron is being prepared.

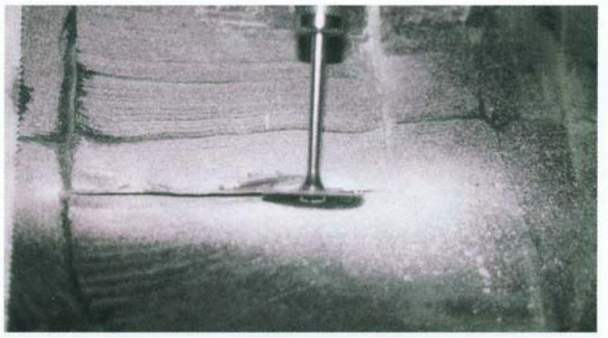

Fig. 15.28 Cutting through the plastron at an angle makes the inner aspect of the flap smaller than the outer.

This ensures that, once replaced, the flap will not fall into the tortoise and can be easily fixed back in position.

This author (SM) tends to cut three sides and score the fourth caudal flap edge as a potential hinge.

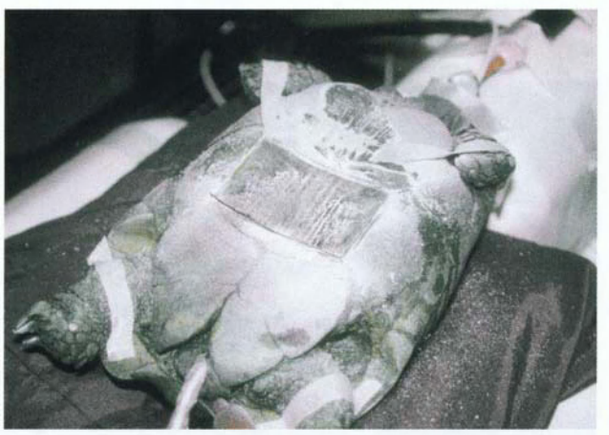

Fig. 15.29 A great deal of powdered debris is created by the burr and the plastron will need to be cleaned again prior to surgical draping.

It is crucial to remove all possible debris and prevent it entering the coelomic cavity.

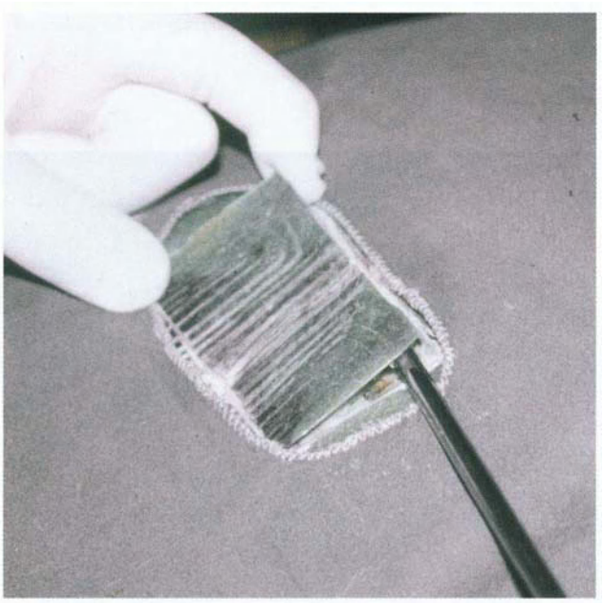

Fig. 15.30 Initially this author (SM) tends to use a sterile orthopaedic screwdriver, or on osteotome, or both, to lever open the osteotomy flap.

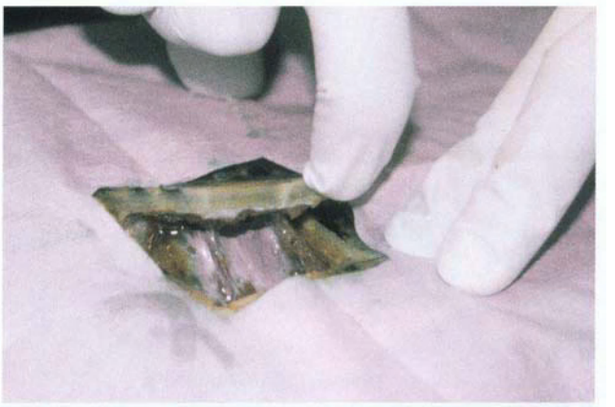

Fig. 15.31 Once the three incised sides of the flap have been freed using the lever, the flap is easily raised using fingers. It will be necessary to use blunt dissection on soft tissue attachments and wherever possible care should be taken to avoid trauma to blood vessels or the coelomic membrane itself.

Fig. 15.32 Here one of the paired coelomic vessels has become traumatised during flap elevation. The vessel is clamped at each end and then ligated to reduce the possibility of perioperative haemorrhage.

The coelomic membrane is incised in the midline or lateral to the blood vessels. The coelom is entered. Gelpi retractors can be used to maintain suitable exposure. As the membrane is reflected, the epigastric vessels, the bladder and its coelomic membrane attachments and the pericardium and its coelomic membrane attachments are all protected and maintained.

Having entered the coelomic cavity, the contents are more fully revealed, allowing a visual survey. The bladder and pericardium should be identified and avoided during the surgical procedure, unless specific entry is desired. These are very delicate structures and if they are subjected to any inadvertent trauma every effort should be made to repair it. The contents of the bladder are seldom sterile and therefore any spillage of urine into the coelom will necessitate cleansing.

Coelom closure

It is crucial to avoid unnecessary trauma wherever possible when closing the coelomic membrane. In the majority of plastron

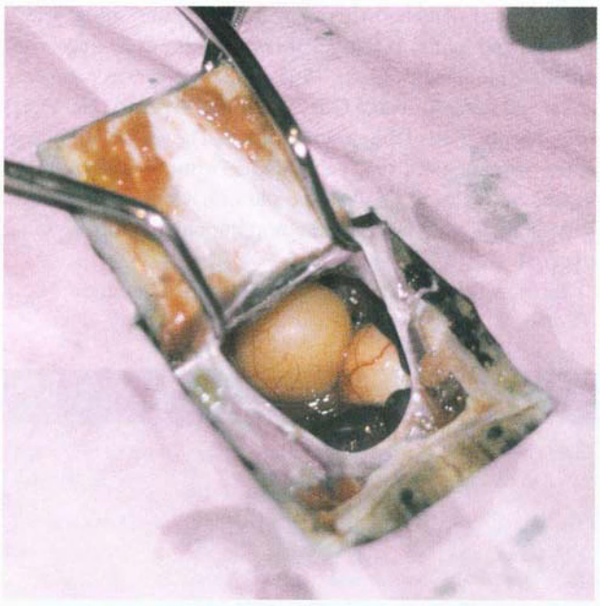

Fig. 15.33 The coelomic cavity is incised in the midline. Gelpi retractors are used here to reflect the membrane laterally and provide suitable access. The epigastric vessels are preserved where possible.

Fig. 15.34 The coelomic contents are revealed. The pericardium, gall bladder and urinary bladder can all be identified and protected. They are delicate structures prone to damage with serious complications, as a result of rough handling.

Fig. 15.35 Maintaining soft-tissue attachments may increase the viability of the flap and speed healing. Here muscle and soft tissue attachments to the caudal edge of the osteotomy flap have been left undisturbed.

Fig. 15.36 The coelomic membrane has been sutured with simple interrupted sutures using 3 metric PDS II ® (Ethicon, Johnson and Johnson International).

The paired coelomic vessels have been preserved.

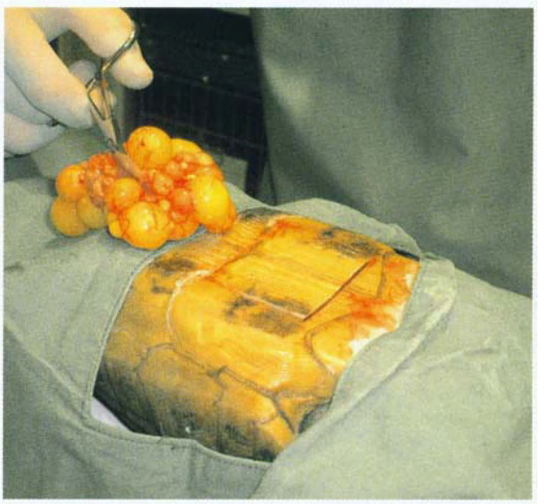

Fig. 15.37 It is clear from Fig. 15.36 that the inner aspect of the osteotomy flap is smaller than the outer. This means that it fits snugly in place and will not fall into the ani—al when the flap is replaced.

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osteotomies, osteogenesis occurs primarily from preserved coelomic membrane, and the osteotomy flap seldom survives. In effect, it is an ideal natural bony bandage allowing healing to occur beneath it. In the case of some semi-aquatic species, with proteinaceous shells, the osteotomy may be better discarded, as discussed later. In terrestrial species, the coelomic membrane is apposed and sutured using an absorbable monofilament material such as 3 metric PDS II® (Ethicon, Johnson and Johnson International). The plastron flap is reflected back into position to cover the surgical defect.

Flap closure

Fibreglass or other coverings are temporary protection until the animal heals its osteotomy injury. Where protective flaps become

dislodged, the need for their replacement will depend upon the strength of the underlying tissues. In many species the underlying tissues may be able to cope without protection after about six weeks. The bony flap may be shed when underlying tissues have adequately healed. This may be a year or more post surgery.

When using fibreglass patches and epoxy resin, some authors suggest that it is important that material used in sealing the coeliotomy site is not allowed to fill the fissure between the flap and the intact plastron, as, theoretically, this may result in a non-union. This is easily prevented by filling the fissure with a sterile, water-soluble material (e.g. K-Y Lubricating Jelly®, Johnson and Johnson) (Fig. 15.38) or amorphous hydrogel containing a modified carboxymethyl cellulose polymer, propylene glycol and water (IntraSite Gel®, Smith and Nephew) (Fig. 15.52) before application of the closure material. The gel can be impregnated with antibiotic (e.g. ceftazidime: Fortum® Glaxo). It may be also beneficial to roughen the site with sandpaper and clean it with a solvent, prior to the application of resin or other material.

Table 15.12 describes different flap-closure techniques.

Table 15.12 Flap closure.

Fibreglass and epoxy resin	*Resin should be handled in a well-ventilated area to reduce potential intoxication, as most are potent 'psychoactive' solvents.*
(Figs 15.38–15.43)	Layered fibreglass sheets, which may be autoclaved, are stuck over the flap site using an epoxy mixture. Resin and hardener are mixed together in a disposable vessel. • After appropriate cleaning and drying, a layer of resin and hardener is applied to the plastron. • This author (SM) tends to paint the mesh or fibreglass with epoxy resin on a protected surface. • Sticky sheets can then be applied directly onto the animal. This reduces resin dribbling across unwanted areas. Alternatively, zinc oxide tape can be used to mask off areas where the resin should not be allowed to go. • The defect is then covered in several layers of resin material and the temperature of the setting material monitored (the reaction is mildly exothermic). • Heating does not seem to be a major problem. • The ratio of hardener to resin will affect setting time and heat production. • The tortoise is kept in dorsal recumbency until the fibreglass holds the flap, or the flap is supported with a dressing until the resin is hard. • The animal can be supported whilst the resin sets, with the flap prevented from making ground contact (disposable cups and pill bottles have been used) (Figs 15.42–15.43). If the surface is sticky while the resin hardens, it can be covered with tape strips like Durapore® (3M), which can easily be removed later at any time.
Hoof resin, Technovit®, dental cement, bone cement, superglues, resins	Materials other than fibreglass can be used to repair the plastron osteotomy site. These can be mixed and piped over the osteotomy site using a large-bore syringe. • Cyanoacrylate ester (Superglue®, Loctite Corporation) was advised by Barten (1996) as an aid to maintaining bandage and dressings in aquatic and semi-aquatic species. This type of acrylic glue has also been used temporarily to retain plastic/cellophane/shrink-wrap plastic coverings over injuries by this author (SM). • Epoxy resins such as Araldite® (Ciba) are tough and waterproof, and bond well to keratin. • Cement-like material such as Technovit® can be used as a mouldable 'gasket' to stabilise an osteotomy flap. Here, following adequate closure of the coelomic membrane, a soft tube/length of cement material is applied to the edge of the plastron incision and the flap is pressed into it before the cement has been allowed to set. The flap is supported and sealed by the ring of cement.
Stainless steel sutures, screws or arthrodesis wires	Stainless steel sutures, screws or arthrodesis wires are used to hold the osteotomy flap stable. Cements, glues or other materials are then used over the fixation to protect and seal it.
Polytetrafluoroethylene (PTFE) Gore-Tex Mesh®	PTFE is helpful where the animal is very large or resides in water, which complicates the use of a fibreglass patch. It has been used historically to repair a plastron osteotomy site following wound breakdown resulting from infection and water exposure. A synthetic, non-absorbable, PTFE Gore-Tex mesh® was successfully applied to a fistulated and infected plastron osteotomy site of a green sea turtle (*Chelonia mydas*), following the failure and degenerate infection of a previous coeliotomy closure using a fibreglass patch. Linear polypropylene was used as an outer covering to encourage epithelialisation and the site was protected with petroleum jelly and waterproof tape. The turtle was maintained out of water for a month before successfully being reintroduced into a hospital swimming area (Mader 1998d).
Shrink-wrap plastic	Shrink-wrap plastic coverings used in the retail and food industries are ideal dressings to retain osteotomy flaps in species where regular dressing changes are necessary. Generally, such coverings conform to the shape of the animal in response to heat applied by a hairdryer or similar device. These materials suit semi-aquatic and high-humidity species where fibreglass patches are often poorly tolerated. They are also water repellent if utilised with waterproof tape as described earlier.
Metal sutures, synthetic sutures and screws	PDS sutures, stainless steel sutures, screws and orthopaedic or arthrodesis wires have been used to support the plastron osteotomy bone flap (Fig. 15.45). These materials are generally used in combination with some other form of wound dressing or protection.

Post-operative care

Following a plastron osteotomy, consideration must be given to analgesia, antibiosis, fluid therapy and the provision of a recovery environment vivarium. Most animals benefit from a short period of hospitalisation until the risk of complications has passed (up to two weeks).

Complications following coeliotomy

Table 15.13 summarises the possible complications following coeliotomy.

Resolution of an infected plastron osteotomy site in a *Terrapene* sp. is illustrated in the accompanying plates (Figs 15.48–15.56). In this case, repair was facilitated by the use of amorphous hydrogel containing a modified carboxymethyl cellulose polymer, propylene glycol and water (IntraSite Gel®, Smith and Nephew), a dry dressing (Rondopad®, DEWE+Co) and, later on, a perforated radiography film as a dressing.

Fig. 15.38 The osteotomy fissure is packed with a water-soluble material (e.g. K-Y Lubricating Jelly®, Johnson and Johnson) or amorphous hydrogel containing a modified carboxymethyl cellulose polymer, propylene glycol and water (IntraSite Gel®, Smith and Nephew) before application of the closure material.

No data is available to suggest this may interfere with healing.

Table 15.13 Complications following coeliotomy

Infection and poor healing environment	The detrimental effects of inadequate environmental temperature, excessive or inappropriate water exposure and humidity, prior infection of the patient, inadequate environmental hygiene and poor levels of nutrition upon wound healing have already been discussed at the start of this chapter. It would seem that the coelomic membrane, and material below it, has a tendency to become necrotic when exposed to a damp and bacterially contaminated environment (as when swimming) (Fig. 15.48). A soft-tissue flank approach may therefore be preferred in aquatic and semi-aquatic species. Advice concerning the coeliotomy approach of choice has been provided earlier in order to try to reduce such complications wherever possible. Mader (1998d) and Bonner (2000) describe complications in the healing of surgical and traumatic injuries of the carapace and plastron in both aquatic and semi-aquatic species. Bonner (2000) points out that high-humidity and swimming species, which tend to shed scutes (e.g. painted turtles, *Chrysemys* spp., sliders, *Trachemys* spp. and cooters, *Graptemys* spp.) are poor candidates for application of fibreglass patches. So are species that peel portions of keratin on the scutes (e.g. *Chelydra* spp. and *Kinosternon* spp.) during growth. For these species, the osteotomy flap may be best discarded after a short period of bandaging *in situ*, whilst the coelomic membrane stabilises. Wet-to-dry bandaging or application of shrink-wrap plastic may be more appropriate forms of treatment than application of fibreglass (Figs 15.48–15.56). Placement of water- and humidity-loving animals into soil or water is not advised in the immediate post-operative period following a plastron osteotomy. These animals are best 'dry-docked' or housed out of water during the first few post-operative weeks. Misting with water from hand sprays, damp, regularly changed towels and the use of towelling as substrate, and decreasing plastron contact with wet surfaces (e.g. plastic draining board matting as described earlier) are more suitable. If the animal must feed in water, placement of an oesophagostomy tube may be necessary, or the animal may be allowed to swim in clean water for a short period once the coelomic membrane has sealed. Marine chelonians can be kept in shower units and showered regularly.
Bladder rupture and pericardial trauma	Great care must be taken to avoid unnecessary trauma to the fluid-filled bladder and pericardium during incision of the coelomic membrane and during endoscopic exploration of the coelomic cavity. Digital stimulation of the cloaca will often trigger reflex urination in the conscious patient prior to induction, reducing the possibility of trauma or of contamination of the coelom in the event of penetration. Bladder contents must be considered to be heavily contaminated with faecal organisms. Release of bladder fluid into the coelomic cavity is an indication for extensive cleansing. Any traumatic bladder defect must be repaired (cystotomy is discussed later in this book). An un-repaired bladder rupture will result in coelomitis and metabolic imbalances associated with urocoelom (e.g. hyperkalaemia and hyperuricaemia).
Haemorrhage	Care should be taken to ensure that all vessels associated with visceral surgery and the vessels of the coelomic membrane are adequately ligated or sealed during the course of a surgical procedure (Fig. 15.32). • It is possible that changes in blood pressure and vascular tone associated with anaesthesia will mean that sites where intra-operative haemorrhages were not apparent during surgery may bleed profusely later in recovery. Such patients may benefit from administration of colloid plasma expanders, oxyglobin or blood transfusion, as described earlier in this book. • Where the surgeon suspects that post-operative haemorrhage may still be occurring, repeat exploratory surgery and ligation of the coelomic or other vessels may be worthwhile.
Growth	Where plastron closure occurs on growing individuals, careful monitoring of the surgical site and regular replacement of restrictive closure materials may be necessary.

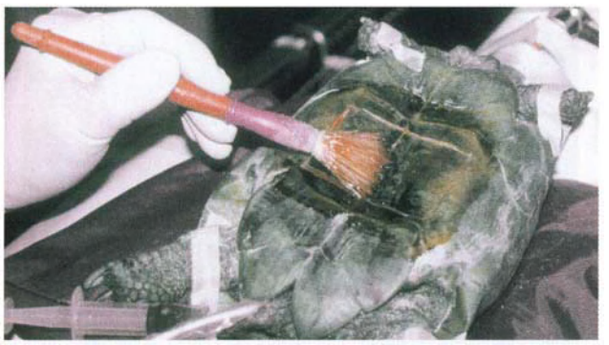


Fig. 15.39 The plastron should be clean and dry before application of the first coat of epoxy resin and hardener mix. Care should be taken to prevent excess material dribbling over the animal.

Fig. 15.40 Layers of fibreglass are cut to size, moistened with epoxy resin and then applied to the plastron.

Fig.15.41 The defect has now been adequately covered. It will take several hours for the material to set and the reaction will produce a moderate amount of heat.

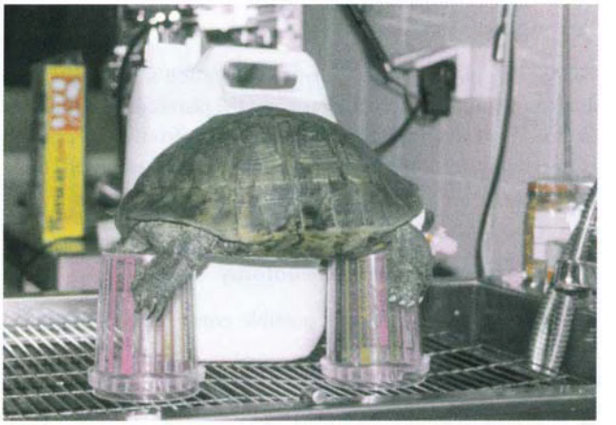


Fig. 15.42 The animal is placed back into ventral recumbency at the earliest opportunity so that lung volume is no longer compromised by the weight of the coelomic viscera.

After initial setting of the material it can be covered with an easily-removable tape, like Durapore® (3M), to prevent sticking to the ground when the animal is brought back into its normal orientation.

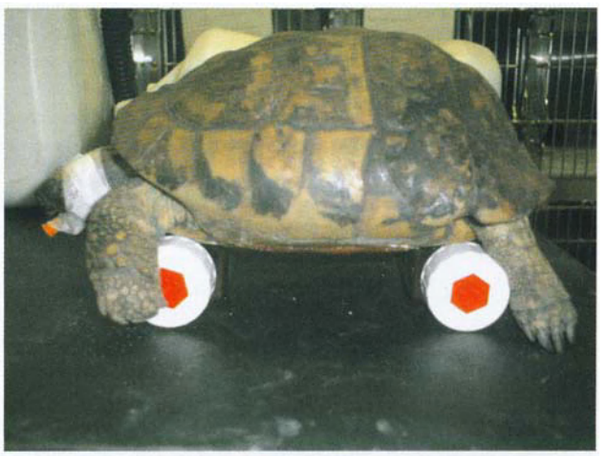


Fig. 15.43 Here pill bottles placed either side of the fibreglass-and-epoxy matting support the animal's weight.

Fig. 15.44 It is possible to apply very simple dressings with tape to facilitate healing of an osteotomy flap. Here a ventilated section of a Rondopad® (DEWE+Co) has been removed and taped over the wound to protect it and allow drainage. Tegaderm™ is also suitable.

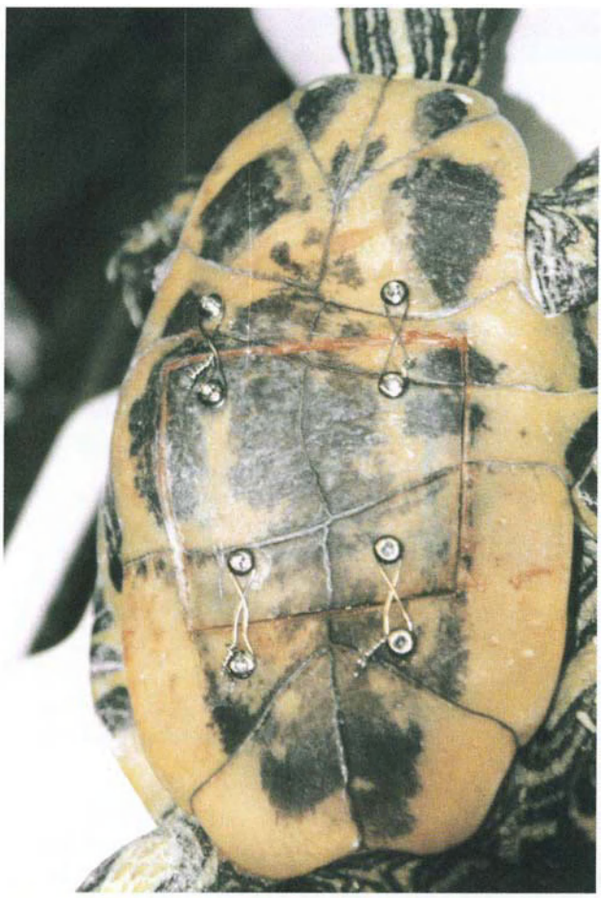


Fig. 15.45 Screws were used in the reduction of this osteotomy. This union can then be covered by a small amount of hoof cement covering the fissure and implants.

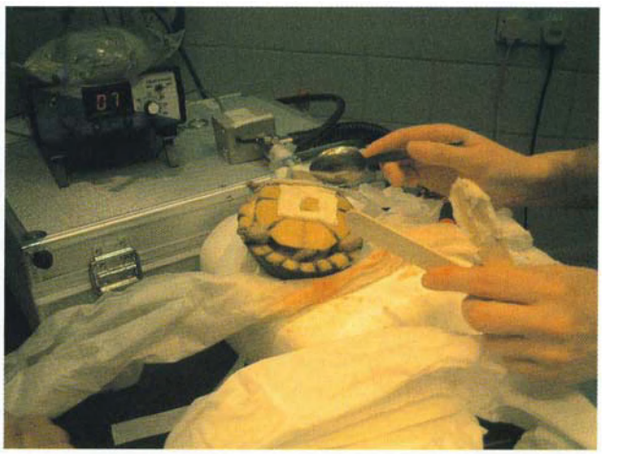


Fig. 15.46 Here methylmethacrylate is used as a gasket to support an osteotomy flap in a box turtle (*Terrapene* sp.). An alternative method of application is to pipe it like icing sugar.

Fig. 15.47 Removal of the fibreglass patch nine months after surgery reveals dead bone overlying a healed and regenerating plastron. Healing appears to come from osteogenesis at the surface of the coelomic membrane.

PREFEMORAL/SOFT-TISSUE FLANK APPROACH

Indications

A soft-tissue flank approach allows unilateral access to the small intestine, bladder, ovarian and oviductal tissue and liver. Exposure of the intestine through a soft-tissue flank approach, just cranial to the hind limb in the red-eared slider (*Trachemys scripta elegans*), was well described by Brannian (1984) and later by Gould *et al.* (1992). Other procedures described include hemiovariosalpingectomy, foreign-body removal, liver and kidney biopsy and cystotomy. The endoscopy section of this book already explains how virtually any coelomic organ can be examined and a biopsy taken through a relatively atraumatic small incision in this area.

Coelomic procedures possible through a prefemoral approach

Table 15.14 describes the procedures commonly carried out through a prefemoral approach.

Fig. 15.48 This box turtle was presented 3 weeks post coeliotomy. The flap had been repaired using a fibreglass patch and epoxy resin. There was a foul discharge coming from beneath the patch, which was removed under general anaesthesia. Antibiotics, analgesics, and other supportive care were provided.

Table 15.14 Procedures possible through a prefemoral/soft-tissue flank approach.

Endoscopic examination	• Coelomic endoscopy is covered in detail in the Endoscopy section of this book. • Care should be taken to identify the bladder wall and avoid traumatic entry. • An endoscopic examination (exploratory laparotomy) may assist in determining the need for greater access, e.g. through a temporary central plastronotomy.
Renal biopsy	• Endoscopic procedure is described elsewhere as is a trans-carapacial drill-hole and needle aspirate. • The coelomic membrane does not require incision. • The kidneys are generally caudodorsal from the incision site. If the prefemoral plastron is very wide, or the prefemoral space very narrow, it might be difficult to pass the endoscope dorsolaterally enough to visualise large parts of the kidneys. Sometimes they are massively enlarged and can either be palpated or visualised using ultrasonography. • Only one kidney can be reached from each side. • The kidney is reached through burrowing and blunt dissection of the coelomic membrane away from the caudodorsal carapace. • Care must be taken to avoid penetration of the septum horizontale and damage to lung tissue. • Following immobilisation and biopsy (a biopsy punch can be used) an adrenaline swab can be used to stem bleeding. Alternatively, a suitably-sized Gelfoam® (Upjohn Company of Canada, Don Mills, Ontario) foam pad can be wedged into areas to control haemorrhage. Haemorrhage is unusual with endoscopy equipment smaller than 5FG. • Soft tissues are closed down eliminating dead space using a monofilament material (e.g. PDSII®, Ethicon) as described earlier.
Liver biopsy	• Liver biopsy is relatively easy through a flank approach. • It should be remembered that the liver is responsible for the production of some clotting factors, and that patients with a severe hepatopathy may have subnormal clotting. The laboratory assessment of coagulation pathways is described in Clinical Pathology. • Endoscopic biopsy is described elsewhere. • Non-endoscopic biopsy can be performed with the aid of a sterile otoscope. • Blind biopsy using forceps is also possible but carries a risk of complications. The liver is generally friable, especial when pathology such as lipidosis is present. Grasping hepatic tissue with forceps is therefore likely to result in partial hepatectomy, which may in turn result in haemorrhage that is difficult to control. • Haemorrhage from most liver biopsy and hepatectomy sites is usually short-lived, and in the author's experience rarely compromises the patient.
Ovariosalpingectomy	• Hemiovariosalpingectomy was successfully performed by Nutter *et al.* (2000) in a 118 kg loggerhead sea turtle (*Caretta caretta*) through a lateral flank approach, following prolapse and resection of an oviduct by a referring veterinarian in the field. • The animal appears to have been returned to water within 24 hours. The animal nested two years later and laid fertile clutches of eggs from the remaining ovary and oviduct. • It was hypothesised that balance and buoyancy may have been affected by uneven distribution of ova and eggs, but this was not observed. • Bilateral incisions may be required.
Salpingotomy	The contralateral oviduct may not be available and dystocia therefore cannot be guaranteed to be surgically resolved unless bilateral incisions are made. A central plastronotomy is generally preferable.
Prolapse reduction	Not advisable as access is likely to be inadequate. A central plastronotomy is generally advisable, although colopexy to the body wall can be used to retain a rectal prolapse.
Enterotomy	• Small foreign bodies can be removed by this approach but it must be remembered that radio-opaque material in the digestive tract may be an incidental finding. • Contrast radiography may suggest intestinal obstruction but choice of media may affect suitability for intestinal surgery (see radiography). Care should be taken to check for concurrent disease that may otherwise explain anorexia. • Brannian (1984 & 1992) and Gould *et al.* (1992) advise the use of a spay hook to exteriorise loops of intestine (small animals less than 2 kg). • Gould *et al.* (1992) performed a linear enterotomy to remove impacted shell fragments in a 500 g red-eared slider. • Isenbugel & Barandun (1981) removed an obstructive rubber plug from a 15 kg turtle at Zurich Zoo.
Cystotomy	For detailed technique, see Table 15.20 in the Cystotomy section, below.

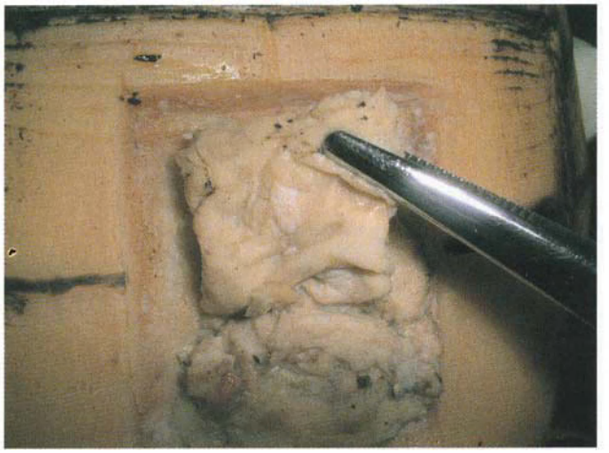


Fig. 15.49 Caseous material filled the defect between the coelomic membrane and the osteotomy flap.

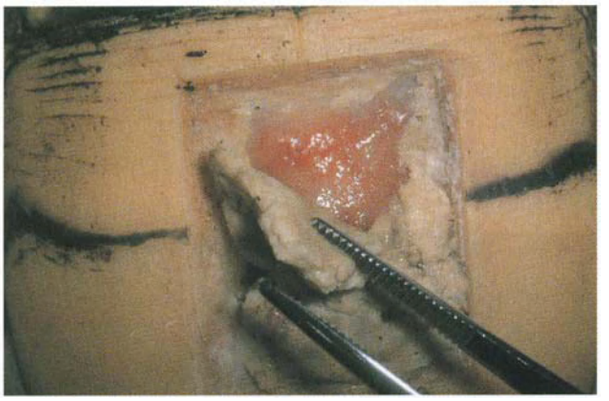


Fig. 15.50 Necrotic material was gently removed using surgical implements and blunt dissection.

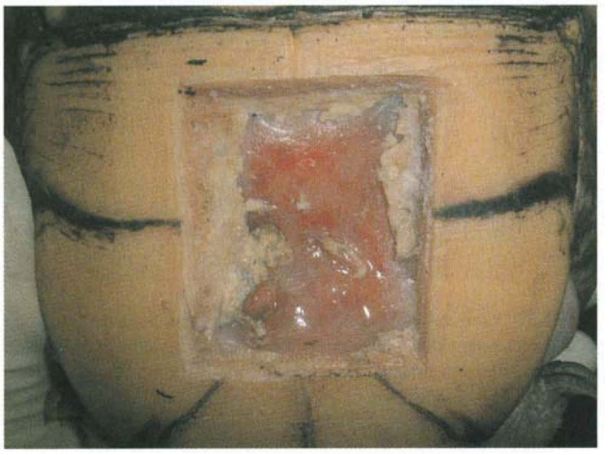


Fig. 15.51 A healthy granulation bed lies below the caseous necrotic plug. The coelomic membrane has hypertrophied and is actively repairing the defective plastron.

Technique

The animal is stabilised and given appropriate pre-operative fluids, analgesics and antibiotics as discussed elsewhere in this text. Table 15.15 describes the procedure.

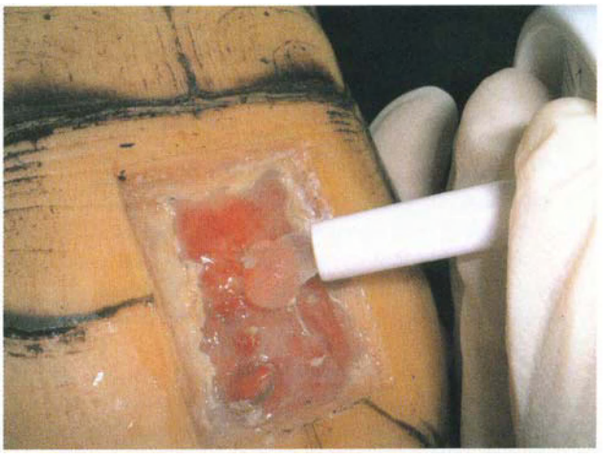


Fig. 15.52 The wound is cleaned and protected using amorphous hydrogel containing a modified carboxymethyl cellulose polymer, propylene glycol and water (IntraSite Gel®, Smith and Nephew).

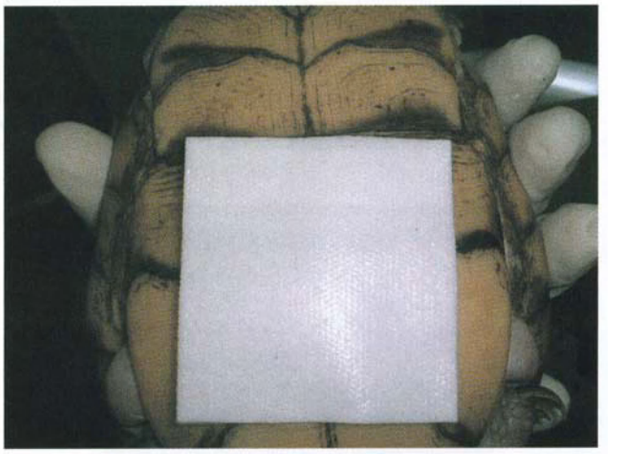


Fig. 15.53 A dry dressing (Rondopad®, DEWE+Co) is applied. The osteotomy flap has already been discarded.

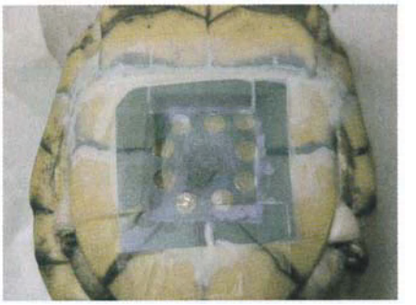


Fig. 15.54 As the surgical wound stabilises and healing begins, a protective covering is made using radiography film. A hole-punch has been used to created ventilation points. Simple surgical tape holds the film in place. This animal is 'dry-docked'.

LATERAL PLASTRONOTOMY COMBINED WITH PREFEMORAL APPROACH

This technique, in which a small hinge is created in the lateral plastron in order to increase exposure, is suitable for all chelonians. This approach has benefits over a more central

Table 15.15 Pre-femoral (soft-tissue flank) approach.

(1) The animal is anaesthetised and placed in lateral recumbency (with the cranial carapace higher than the caudal carapace to assist with cranial lung inflation).
(2) The chelonian can be immobilised by placing it in a purpose-built cradle, or shaped plastic bottles, and other surgical supports can be used as described earlier.
(3) The side to be entered surgically will depend on the anticipated procedure and diagnostic data. Occasionally it may be necessary to enter both sides.
(4) Brannian (1984) and other authors suggest that the incision site is best exposed with the ipsilateral leg restrained in extension, e.g. using tape. The other legs should also be immobilised in a manner reducing the risk of accidental trauma.
(5) The surgical site is aseptically prepared.
(6) A cranial-to-caudal skin incision is made in the skin of the middle third of the fossa. Care should be taken, as if the incision is extended too far caudally, the femoral vein in the inguinal region may be cut (Brannian 1992).
(7) The subcutaneous and muscular tissues are incised and parted to reveal the fibrous, white coelomic membrane.
(8) Care should be taken when the coelomic membrane is incised. It can usually be entered using the closed points of a pair of Mayo scissors. In some animals a stab incision with a pointed scalpel may be necessary. The lung fields may be attached to the dorsal half of the membrane covering the fossa. For this reason a central or slightly ventral incision is generally preferred.
(9) The bladder will lie immediately behind the coelomic membrane if distended. Care should be taken to prevent accidental puncture. The contents of the bladder must be regarded as contaminated and it may therefore be best to perform surgery immediately after urination has been observed. Alternatively, it may be possible to pass a Foley catheter into the anaesthetised patient and deflate the bladder prior to flank surgery if ultrasound or other evidence suggests that it is significantly distended.
(10) Following coelomic entry, visual, digital and endoscopic exploration become possible.
(11) Brannian (1984 & 1992) and Gould *et al.* (1992) advise the use of a spay hook to exteriorise loops of intestine (animals less than 2 kg).
(12) Closure is multiple layered. Simple interrupted, horizontal mattress sutures (PDS®, Ethicon) in the coelomic membrane, simple interrupted sutures in the subcutaneous and muscle tissues (PDS®, Ethicon) followed by an everting skin layer of sutures or staples.
(13) Turtles can be returned to water 24–48 hours after soft-tissue coeliotomy closure.

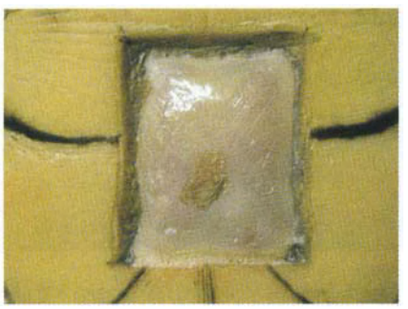


Fig. 15.55 Epithelialisation has started within a week of removing the necrotic material from the osteotomy site.

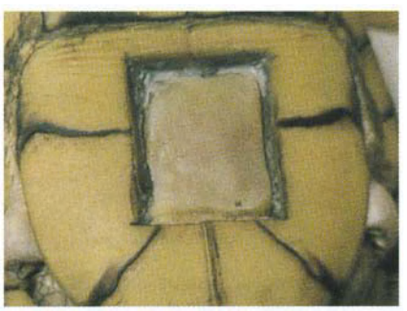


Fig. 15.56 Within six weeks a significant degree of keratinisation has occurred and the animal can have increased exposure to shallow baths and high humidity environments once more.

plastron osteotomy in aquatic and semi-aquatic species because the animal may become waterproof sooner post operatively. It is therefore able to tolerate water exposure earlier, suffers fewer complications and post-operative attention to the osteotomy site is greatly reduced.

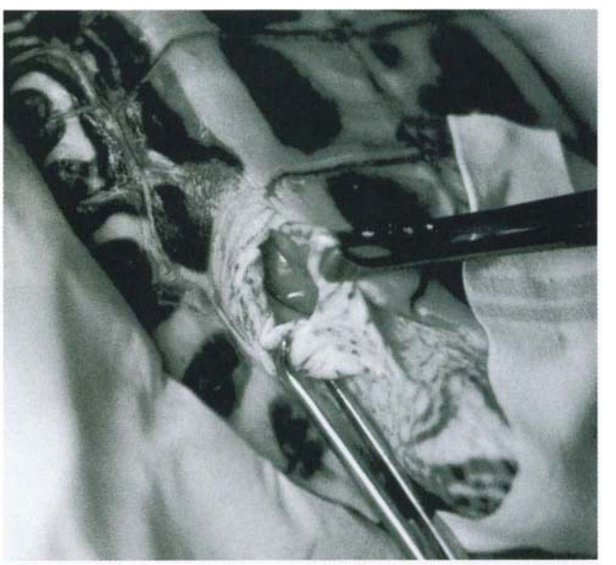


Fig. 15.57 A soft tissue approach in small chelonians, less than 1 kg, such as in this cadaver (*Trachemys scripta elegans*) provides very limited access and exposure and is really only suited to endoscopic examination and biopsy of easily-located viscera (post-morten).

A triangular area is marked out and scored on the lateral plastron immediately beside the pre-femoral fossa using a high-speed burr. The plastron hinge created is easily stabilised by crossed K wires, which can be removed after four weeks. Soft tissues are closed using PDS sutures in layers as described earlier.

Figures 15.57–15.62 demonstrate this procedure carried out post mortem on a 500 g red-eared slider (*Trachemys scripta elegans*). The author stresses that, during ante-mortem surgery, the patient should be appropriately anaesthetised, aseptically prepared and draped, and surgical gloves should be worn.

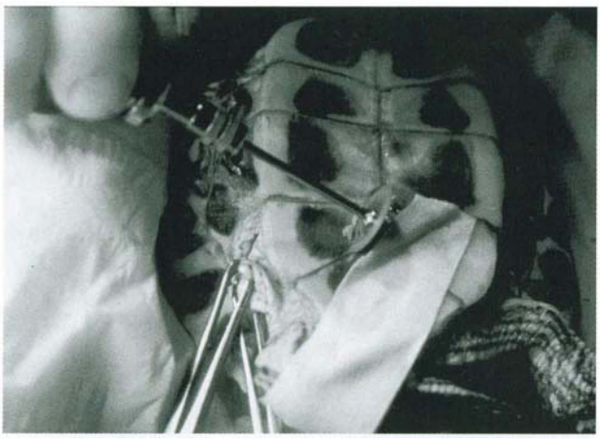


Fig. 15.58 The surgical area, the plastron craniolateral to the inguinal fossa, is marked out and scored. Two plastron incisions are completely transacted at right angles to the plastron edge, along the margins of the femoral scute, to a distance of approximately two thirds of the femoral scute. Protecting limbs by taping them away from the surgical site will reduce the chance of inadvertent trauma.
NB: post-mortem, wear gloves.

Fig. 15.59 A flap hinged around the medial edge is created by merely scoring a third side between the two incisions already described in Fig. 15.58.
NB: post-mortem, wear gloves.

Fig. 15.60 Access and exposure are greatly increased in comparison to Fig. 15.57.
NB: post-mortem, wear gloves.

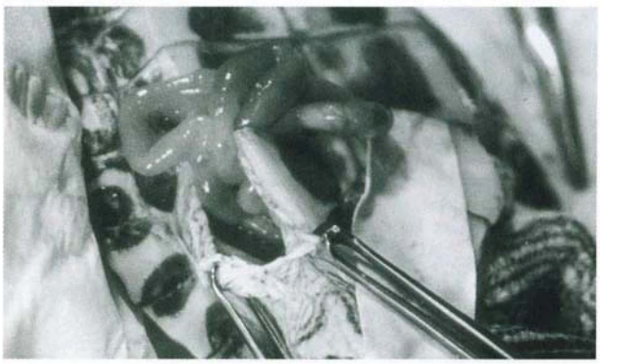


Fig. 15.61 The small intestine is easily exteriorised, allowing examination and surgical intervention. The liver, bladder oviducts and other structures are also made available to varying degrees.
NB: post-mortem, wear gloves.

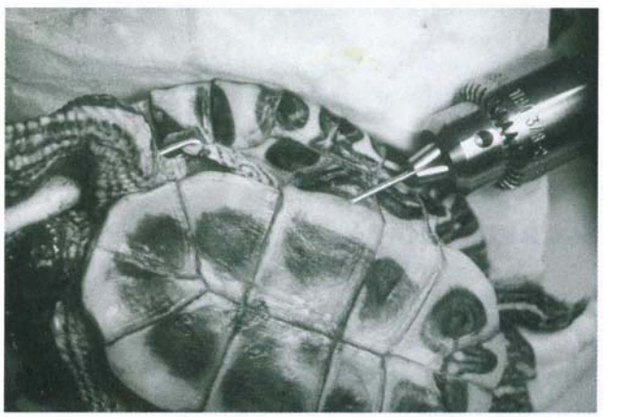


Fig. 15.62 Post operatively, the plastron is easily stabilised by crossed K wires removed after 4 weeks. Soft tissues are closed using PDS sutures in layers as described earlier. Resins and glues are not required.
NB: post-mortem, wear gloves.

OVARIECTOMY

Indications

In the absence of an effective medical-management protocol, ovariectomy currently appears to be the most suitable treatment for follicular stasis in isolated mature captive female chelonians (McArthur 2000a & 2001a). The aetiology and diagnosis of follicular stasis have already been described in the section dealing with a problem-solving approach to disease (Figs 13.44–13.45). Unilateral and/or bilateral ovariectomy and ovariosalpingectomy has also been performed secondary to plastron osteotomy and reduction of oviductal prolapse where the oviduct is considered non-viable by Bennett (1993b). Nutter *et al.* (2000) used a soft-tissue flank approach to perform hemiovariosalpingectomy in a mature loggerhead turtle (*Caretta caretta*).

Technique

The patient is anaesthetised and prepared as previously described. Pre-operative fluids, antibiotics and analgesics are general administered. Table 15.16 demonstrates the technique for ovariectomy.

Table 15.16 Ovariectomy.

(1) The ovaries of terrestrial tortoises are easily exteriorised through a transplastronal osteotomy (Figs 13.48–13.49).
(2) Despite their delicate nature, initial exteriorisation is often a simple procedure involving gentle lifting of the ovaries using tissue forceps in alternate hands. The forceps grasp ovarian tissue between follicles and not follicles themselves (Fig. 13.51). The fingers can also be used very gently (Fig. 13.52). Care must be taken during this procedure not to rupture any follicles, as egg coelomitis will result.
(3) Mesovarium is clamped with haemostats and ovarian tissue isolated (Figs 13.54–13.55).
(4) Care should be taken to ensure that yolk is not allowed to enter the coelomic cavity and all follicles are removed.
(5) Removal of uterine tissue does not appear to be necessary.
(6) Following removal of ovaries the oviducts are allowed to fall back into the coelomic cavity (Fig. 13.56). Removal of oviductal tissue does not seem to be required, although some colleagues do remove it.
(7) The long-term possibility of osteoporosis/obesity following ovariectomy has not yet been investigated.

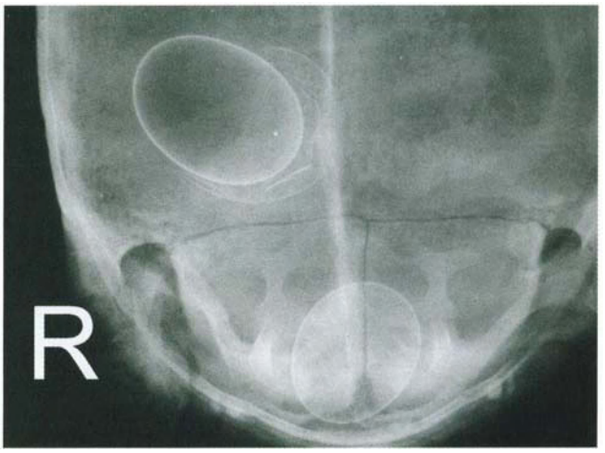


Fig. 15.63 This pelvic egg has obstructed urine and faecal outflow. The oviducts are now full of faecal material and gas. This animal has become toxaemic.

The cranial egg is putrid and full of gas and has become tucked inside the remnants of a second degenerate and necrotic egg. In this case the cloacal egg was aspirated. The further degenerate eggs were let down and aspirated and the oviducts cleared with repeated lavage.

EGG RETENTION

Salpingotomy

Indications

The point at which normality and dystocia diverge is often poorly defined, making chelonian dystocia a complex and subjective diagnosis for a clinician to make. Eggs are retained for some time in normal gravid females in order to allow the production of the calcified shell, so that time elapsed is not a reliable indication.

Aspects of the aetiology, diagnosis and management of dystocia have already been described in the problem-solving section of this book. Here we try to give guidelines as to when medical and surgical intervention might be considered, and suggest an approach to surgery. The techniques described here have been developed in practice. Clinicians are encouraged to alter or adapt advice to complement their own experiences.

Given our current lack of knowledge of captive chelonian reproductive physiology, the indications for salpingotomy (surgical removal of eggs from the oviducts) are poorly-defined and often subjective. The decision to perform salpingotomy often comes down to the knowledge and experience of the clinician concerned. Where the animal has become distressed, or is becoming diseased because of a dystocia, and where these conditions cannot be satisfactorily managed by improvements in care and/or medical intervention, salpingotomy or other surgical intervention may be indicated.

Surgical intervention is an option where medical induction of oviposition has not been successful, or when there is evidence that eggs within the coelomic cavity cannot be delivered through medical induction. Examples of this include ectopic eggs (within the bladder), obstruction (e.g. prolapse) and abnormal size or conformation of eggs. The radiographic appearances of normal eggs, retained eggs, degenerate and ectopic eggs within the bladder are illustrated (Figs 15.63–15.65).

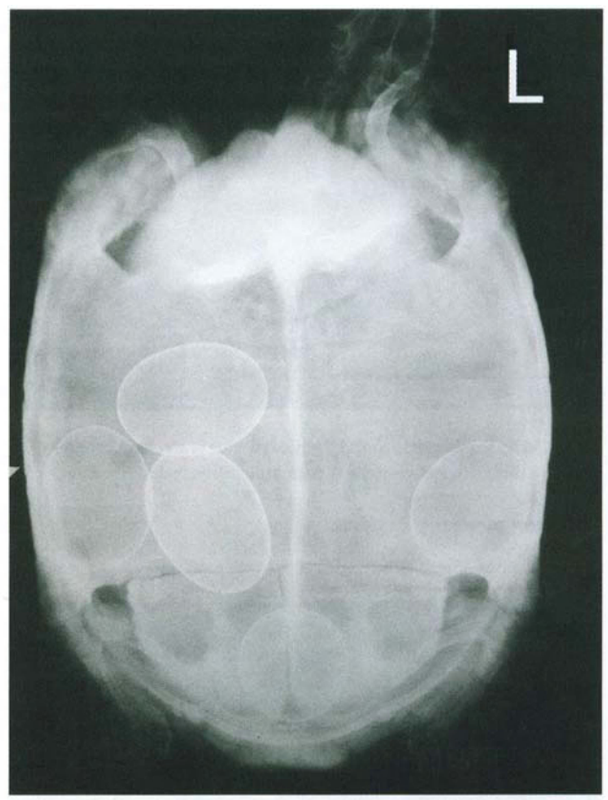


Fig. 15.64 Here dystocia would not resolve with medical induction of oviposition because of a transversely presented, and therefore functionally oversized egg, obstructing the pelvic canal. Following aspiration of its contents by cloacal ovocentesis, this egg was removed.

Occasionally, eggs that have been retained within the shell gland for long periods of time, possibly more than a year, may incorporate folds of the oviductal wall (shell gland) into their shell during the process of calcification. These adhesions can make them impossible to pass, even with medical induction.

Fig. 15.65 These eggs were too large to pass through the pelvic canal but were brought down into the pelvic canal using a combination of oxytocin and atenolol. They were then punctured one at a time with a 19G 1.5" needle, their contents aspirated into a 10 ml syringe and the egg remnants removed through the cloaca using a pair of haemostats. Recovery was then uneventful.

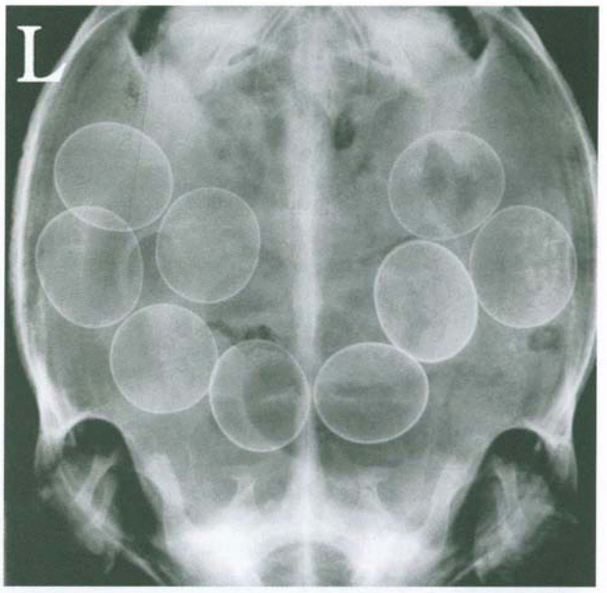


Fig. 15.66 The presence of eggs within the coelomic cavity of a female chelonian does not necessarily mean that the eggs are abnormal and must be removed. Other indications that dystocia is present or that egg production is negatively affecting health are also necessary to make a likely diagnosis of dystocia.

Fig. 15.67 This elongated tortoise (*Indotestudo elongata*) was suffering from relative oversize of eggs. This later became a true dystocia requiring surgical intervention.

In such cases there is only limited evidence of abnormality from clinical investigations such as radiography. Stabilisation, rehydration and medical intervention will be fruitless and a clinician performing an elective salpingotomy will be effectively resolving the dystocia, after an elective decision to do so.

Technique

The patient is assessed and stabilised. Pre-operative fluids, analgesics and antibiotics are administered as necessary. This may involve several days care in a hospital environment. Patient assessment through blood work, monitoring urine output and core body temperature and diagnostic imaging techniques such as radiography (Fig. 15.66–15.67) should already have been undertaken. Table 15.17 describes salpingotomy.

Where a female tortoise has been in the company of a compatible male within the previous three or four years, the eggs from a salpingotomy procedure may be viable. Use of an incubator should be considered, and it is generally wise for the eggs to be returned to the responsibility of the keeper at the earliest opportunity. We often encourage keepers to collect eggs long before they collect the chelonian.

Chelonians can be spayed (ovariosalpingectomy), during salpingotomy. This will prevent further ovulations and so avoid any recurrence of surgical dystocia. This is best discussed with the client prior to commencing surgery, and appropriate consent obtained. Many specimens are endangered species: it may be prudent to avoid spaying these without good reason. It may be possible to suppress unwanted ovarian activity in a proportion of animals using medical protocols such as proligestone as described in the Follicular Stasis section in the problem-solving part of this book.

It is essential to ensure that concurrent health/husbandry problems are addressed. Dystocia is not generally a surgical

Table 15.17 Salpingotomy.

(1) If the patient has not been in the care of the clinician since the diagnosis of dystocia was made, it is wise to ensure that eggs have not been unexpectedly voided (e.g. during transport or hospitalisation). An immediate pre-surgical radiograph confirms the situation. However, if the animal has been hospitalised by the clinician then the enclosure should be thoroughly searched, and a pre-surgical radiograph considered. The pre-surgical radiograph may also help to identify structures to avoid when entering the coelom (Fig. 15.66).

(2) A central plastron osteotomy as already outlined is then performed. The size of the osteotomy must be adequate to allow egg material to pass through, with a margin for tissue handling. This is determined from the pre-operative radiographs.

(3) The coelomic cavity is then inspected. Identification of coelomic viscera is usually straightforward unless a necrotising coelomitis is present or the bladder is inadvertently traumatised during coelomic entry. In such situations, the post-surgical prognosis is relatively poor, although with appropriate cleansing and surgical and medical care recovery is occasionally possible.

(4) The oviducts can usually be found in the two caudolateral quadrants either side of the central bladder. Exteriorisation of oviductal tissue containing eggs is relatively straightforward, using blunt tissue-handling instruments (Fig. 15.68). Sterilised cotton buds can also be used to manipulate organs in the coelomic cavity. The finger holes of ordinary haemostats make excellent supports for lifting and stabilising coelomic eggs. Alternatively, various sizes of sterile metal teaspoons may be useful (Figs 15.69–15.71).

(5) In order to reduce potential contamination of the coelomic cavity, stay sutures can be used to keep the salpinx in the plastronotomy site, and gauze sponges can be placed below. Both will prevent the leakage of possibly contaminated fluid, or the contents of broken eggs, into the coelomic cavity.

(6) Ensure that all eggs in the coelomic cavity and pelvic canal are removed. Check the bladder and the coelomic cavity for ectopic eggs. Make sure the ovaries are examined for evidence of ongoing activity, degeneration or follicular stasis. Failure to remove the ovaries may lead to recurrence or other ongoing problems in some animals and ovariectomy/ovariosalpingectomy should be considered.

(7) Eggs are removed and the oviduct sutured, much as in a mammalian Caesarean operation, using fine PDS II® (Ethicon). Closure may be unnecessary if a salpingectomy has been performed.

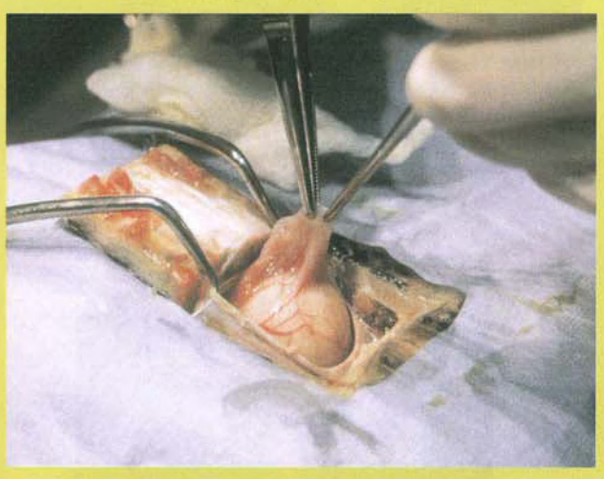


Fig. 15.68 Exteriorisation of oviductal eggs is relatively straightforward using ordinary tissue handling instruments. It is important that the osteotomy is large enough to allow the passage of eggs.

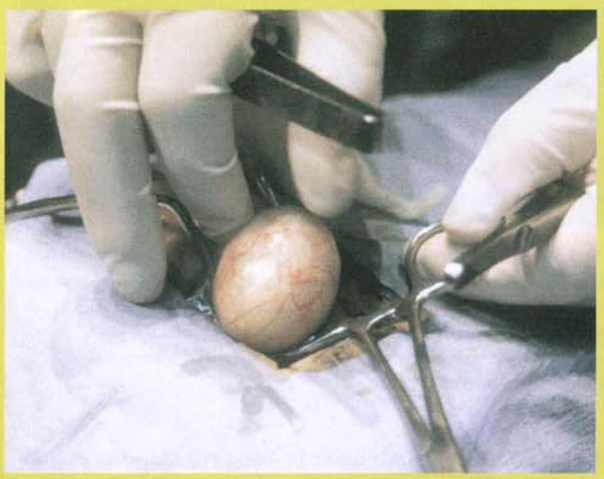


Fig. 15.69 Eggs can be supported using the handles of haemostats or sterile teaspoons. It may be prudent to pack the coelomic cavity with gauze swabs in order to reduce the possibility of contamination if eggs are necrotic. Care should be taken when applying traction to the oviducts to prevent inadvertent trauma to ovarian follicles as this may result in leakage of contents and yolk coelomitis.

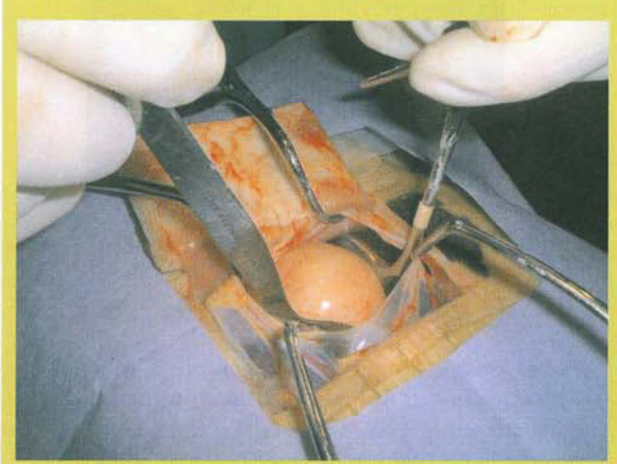


Fig. 15.70 Here a combination of a sterile spoon and the handle of some forceps are used to elevate an oviductal egg.

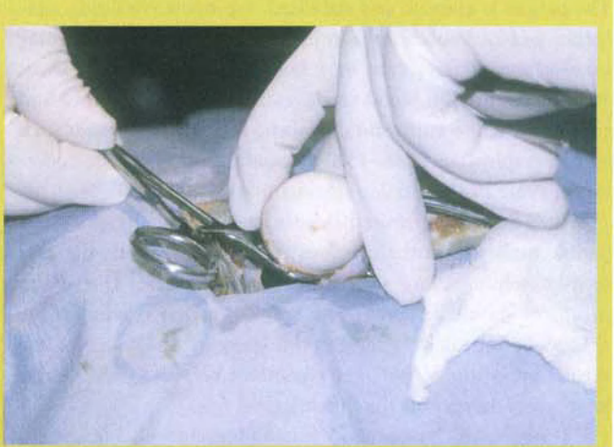


Fig. 15.71 The oviducts are incised, any eggs are removed and the oviduct is sutured. If the chelonian is to be spayed, the oviducts may be removed without first releasing the eggs.

problem and it is important not to miss any other reasons for presentation.

The surgical procedure described above is well described in the literature (Frye & Schuchman 1974; Holt 1979; Rosskopf & Woerpel 1983a; Croce 1984; Müller *et al.* 1989; Brannian 1992; Bennett 1993a; Raiti 1995b; Divers 1997a).

Cloacal ovocentesis

Indications

Cloacal puncture of eggs and aspiration of their contents, as described below, is a realistic and effective option available to the clinician in the management of chronic dystocia, should a keeper decline the offer of coelomic surgery or the animal be deemed too debilitated to cope with it (Rosskopf & Woerpel 1983). Candidates generally have obstructive eggs within the cloaca or at the pelvic brim, but cannot pass them due to oversize. Often eggs are distended with gas and putrid material, which may be apparent using radiography (Figs 15.63–15.65).

Technique

Equipment used by this author (SM) is illustrated in Figs 15.72–15.73. Table 15.18 describes the procedure.

According to Raiti (1995) the presence of oviductal adhesions to shell fragments may still necessitate salpingotomy, although this is unusual. Chronic cases with significant oviductal infection may be candidates for long-term supportive nursing, but should be given a guarded prognosis.

CYSTOTOMY

Removal of large bladder stones or displaced eggs within the bladder may require cystotomy as part of the procedure. Where radiographic and ultrasonographic evidence suggests that an egg is lying within the bladder, coeliotomy and cystotomy to remove this egg is generally necessary to restore health. Figures 15.82–15.83 illustrate two chronically displaced bladder eggs in a *Testudo*. In this case, the animal became hyperkalaemic and hyperuricaemic and died as a result of profound sepsis before surgery was possible. During cystotomy, great care should be taken to prevent non-sterile bladder contents from contaminating the coelomic cavity.

Fig. 15.72 Equipment required for puncture and aspiration of cloacal eggs.

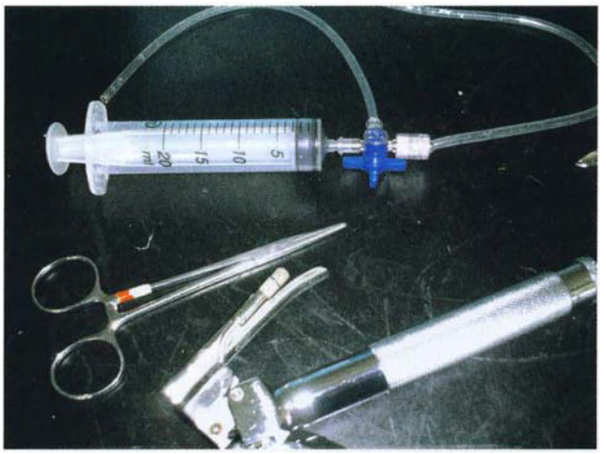


Fig. 15.73 Equipment to debride and irrigate the cloaca and oviducts.

Removal of ectopic eggs

Cystotomy through a central plastron osteotomy is necessary to remove ectopic eggs.

The patient is assessed and then stabilised as described elsewhere. Pre-operative fluids, antibiotics, and analgesics are administered as appropriate. A central plastronotomy is performed (see page 416–427). Table 15.19 describes the cystotomy technique. A similar technique was described by Johnson (2000).

Table 15.18 Cloacal ovocentesis.

(1) A cloacal speculum improves visualisation of obstructed eggs (Figs 15.74–15.76). This author uses a rabbit laryngoscope and rabbit pouch/cheek dilators and oral gags to retract the walls of the cloaca.
(2) For most chelonians, an 18G 2" needle and a 10 ml syringe can be used to aspirate the egg contents (Fig. 15.77).
(3) Aspirated contents are often grossly infected, and if the egg has obstructed faecal and urinary outflow, the cloaca and oviduct may be impacted with both faeces and urine. It is wise to check for evidence of coelomitis at the time of surgery and over the following weeks. This can be done by coelomocentesis and cytology of the aspirate (see Clinical Pathology).
(4) After aspiration of the contents, the egg shell can be crushed and removed in fragments (Figs 15.78–15.79).
(5) The cloaca should then be flushed. A 20 ml syringe attached to a three-way tap and two lengths of drip tube can be used to irrigate the cloaca and shell gland following egg puncture and aspiration (Figs 15.80–15.81). Warm sterile water or saline is suitable for lavage.
(6) Expulsion of debris and encouragement to bring down further retained eggs and uterine detritus is assisted by the use of calcium, β-Blockers, oxytocin and lubrication using doses as described elsewhere in dystocia management. Further cloacal lavage is usually required and the whole process above may need to be repeated over several days depending upon the number of eggs present and the condition of the chelonian.

Fig. 15.74 A laryngoscope allows visualisation of eggs within the cloaca. Oversized eggs can be brought down into the cloaca using the medical induction protocol described earlier in the section describing a problem-solving approach to dystocia.

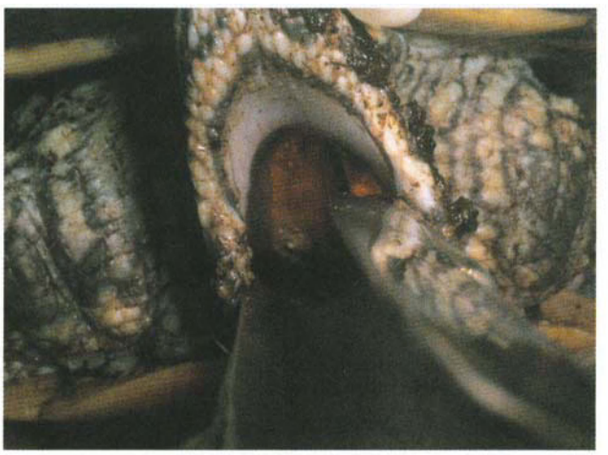


Fig. 15.75 Appearance of a cloacal egg along the blade of a laryngoscope in a red-eared slider (*Trachemys scripta elegans*).

Fig. 15.76 The cloacal speculum allows visualisation of cloacal eggs and facilitates puncture and aspiration using a 19G, 40 mm needle on a 20 ml syringe.

Fig. 15.77 Following egg puncture, the often- putrid contents are easily aspirated.

Fig. 15.78 The now-deflated egg is easily removed in fragments using rat toothed or dressing forceps.

Fig. 15.79 Cloacal removal of chronically retained putrid egg material in *Testudo graeca*.

Fig. 15.80 Cloacal lavage in *Testudo graeca* following removal of a cloacal egg.
A three-way tap attached to a 20 ml syringe and two lengths of drip tubing allows effective irrigation.

Fig. 15.82 Post-mortem dissection of a *Testudo* with two ectopic eggs within its bladder.

Prefemoral approach

The usefulness of this procedure is related to the size of the soft-tissue inguinal opening, and the structure to be exteriorised through it. In many cases there is only limited soft tissue access.

Fig. 15.81 Irrigation of the cloaca in a red-eared slider (*Trachemys scripta elegans*).

Fig. 15.83 The animal in Fig. 15.82 became hyperkalaemic and hyperuricaemic. Death was thought to have been due to severe sepsis.

Tissue handling makes the passage of eggs or sizeable bladder stones unrealistic, and a plastron osteotomy may therefore be necessary. This is especially likely to be the case with small terrestrial species. Table 15.20 describes cystotomy through the prefemoral approach.

ENTEROTOMY

Enterotomy is generally performed where obstruction of the digestive tract is present or suspected, as a result of behavioural alterations, after observation of foreign body/substrate ingestion, in response to medical treatment and management changes, or as a consequence of diagnostic imaging and other investigations.

Table 15.19 Cystotomy to remove ectopic eggs.

(1) The visceral organs are identified following incision of the coelomic membrane. The bladder is generally obvious, especially if distended with urine. It generally lies directly below the osteotomy site in the sagittal caudoventral coelomic cavity.
(2) The coelomic cavity is packed with gauze swabs (or similar) and the bladder is then gently elevated into the osteotomy site and anchored to the coelomic membrane using stay sutures. This stay suture allows marsupialisation and access to the bladder contents with minimal contamination of the coelomic cavity (Fig. 15.84). Tipping the animal may help with egg exteriorisation and decrease possible coelom contamination.
(3) Allocating sterile and contaminated kits and performing a re-prep when returning to sterile surgery after a dirty procedure has been performed is essential. Surgical gloves are of great use as they are simply changed.
(4) A sterile needle, syringe, three-way tap and lengths of drip tubing are often helpful when aspirating bladder fluid and other material from the bladder, because contaminated matter can be relayed away from the surgical site. The technique is similar to that illustrated for lavage with cloacal ovocentesis and allows the bladder to be at least partly deflated before surgical entry.
(5) Following aspiration of the contents of the bladder, it is also possible to aspirate some of the contents of any egg material within it, using a wide-bore needle and a syringe (Fig. 15.84). The egg should, however, be handled and removed complete if surgical access allows. Egg deflation reduces surgical complexity, but the coelomic cavity must be adequately protected from contamination.
(6) The bladder is opened, preferably utilising marsupialisation as already described, and the egg material located and exteriorised. Sterilised spoons and the finger holes of surgical implements are helpful in stabilising and handling eggs. Alternatively, soft-shelled eggs, or crushed eggs, can be grasped and removed with tissue handling instruments (Figs 15.85–15.86).
(7) The bladder is sutured in two layers using PDS II® (Ethicon) sutures. The first layer is interrupted sutures; the second layer is generally continuous and oversews the first. Check for and seal any potential areas of leakage by instilling sterile saline into the bladder (Fig. 15.87).
(8) Care should be taken to observe regular normal urination post operatively.

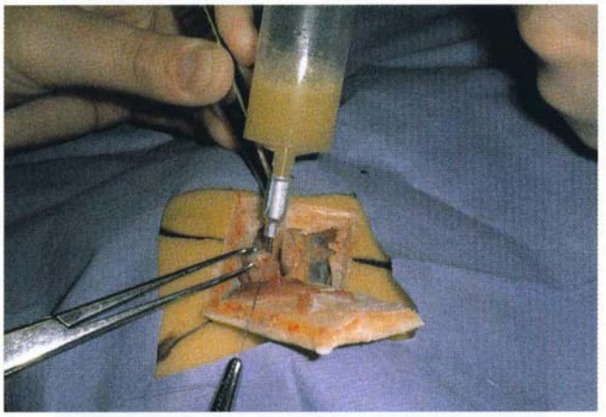


Fig. 15.84 *Terrapene*: The coelomic cavity has been packed off with swabs. The bladder is supported by a combination of sutures anchored by the weight of haemostats and Allis' tissue forceps and then marsupialised. With the bladder partially deflated, egg contents are being aspirated from within the bladder using a needle and syringe.
NB: This author would advise surgical gloves to be worn

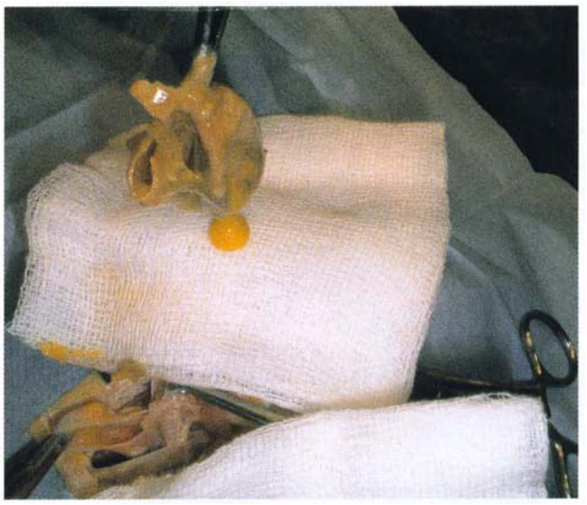


Fig. 15.86 Solid egg material is removed in its entirety. In uricotelic species it may also be advantageous to lavage and remove any urate deposits from the bladder if surgical access and time allow.
NB: This author would advise surgical gloves to be worn

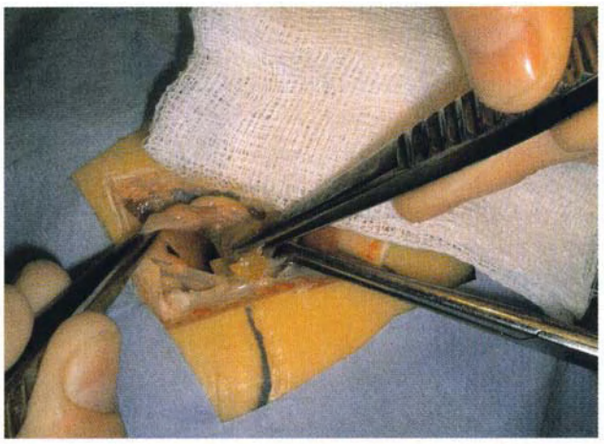


Fig. 15.85 In this case the egg is soft and deflated and grasped relatively easily using tissue-handling forceps. Great care must be taken to reduce the risk of coelomic contamination from bladder contents.
NB: This author would advise surgical gloves to be worn

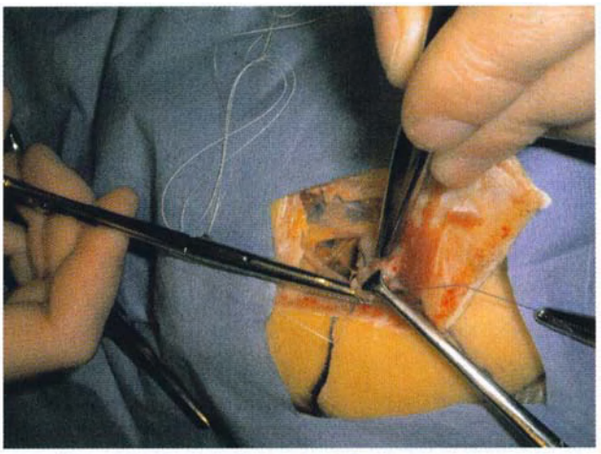


Fig. 15.87 The bladder is closed in two layers and care taken to ensure there are no leakage points.
NB: This author would advise surgical gloves to be worn

Table 15.20 Cystotomy through the prefemoral approach.

(1) The inguinal soft tissues are entered in a soft-tissue approach to the coelom through the prefemoral fossa (see page 427).
(2) The bladder is located and then gently exteriorised using blunt tissue-handling instruments. Great care must be taken to avoid traumatic punctures or tears.
(3) Stay sutures and marsupialisation are beneficial in reducing potential contamination of the coelomic cavity with bladder contents (Fig. 15.88). Allow 1–2 cm of bladder to protrude through the incision.
(4) The coelomic cavity is packed with gauze swabs if access allows.
(5) Some authors have described the crushing, collapse, and removal of ectopic eggs and bladder stones using this approach. As structures within the bladder tend to fall to the most dependent area, long handled Doyen intestinal clamps or curved, sponge-holding forceps may be helpful in reaching them. Alternatively, spoons may be useful.
(6) Following surgery, the site is cleaned using copious fluid lavage.
(7) The bladder is then closed as described above.
(8) Marsupialising stay sutures are removed, soft tissues are closed and the skin is closed.

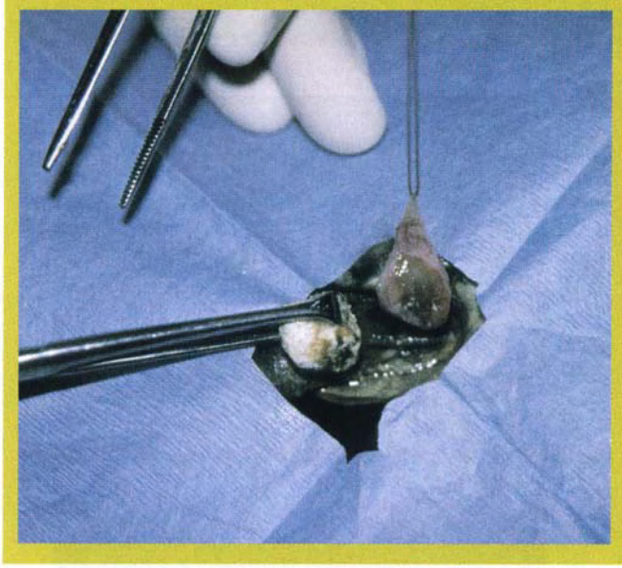


Fig. 15.88 Cystotomy and removal of a bladder stone through a soft-tissue approach through the inguinal fossa of a juvenile *Testudo hermanni*.

Chelonians presented following long periods of anorexia are sometimes thought by their keepers to be suffering from an intestinal obstruction. However, in most cases such animals are experiencing chronic disease unrelated to the digestive tract. Evidence of concurrent disease, contrast studies and response to medical therapy determine the true need for surgical intervention. All cases suspected of intestinal obstruction from a foreign body should undergo a complete physical examination, history review, and appropriate clinical pathology investigations and diagnostic imaging examinations. The possibility that foreign material in the digestive tract is normal or incidental must be considered (Fig.15.89). Most foreign material reaching the large intestine will no longer be a health threat, and some may even aid digestion, as described in the Anatomy and Physiology chapter of this book.

Gastric and oesophageal foreign material may be apparent on endoscopic examination. Occasionally impactions and foreign material show up on plain survey radiographs (Bradley 2000) (Figs 15.89–15.90). Where a radiolucent obstruction is suspected, the choice of contrast medium in radiographic studies of the gastrointestinal tract is important. Barium salts should be avoided where future surgery of the digestive tract is anticipated. Amidotrizoate (Gastrografin®, Schering) and barium-impregnated polyethylene spheres (BIPS®) are more suitable (see Radiography chapter). A mixture of water-soluble lubricant jelly and Iohexol (Omnipaque®, Nycomed) may also be effective, provided desiccation of intestinal mucosa through osmosis is prevented by adequate concurrent fluid administration (Fig. 15.91).

Most obstructions of the digestive tract will resolve with improvements in hydration and the administration of lubricants and laxatives, as described earlier. There is little evidence that gut motility can yet be modified reliably using other medications. Impactions occurring as a result of the ingestion of substrate or other material (such as fishhooks, bone, china, peach stones, corn-cob and plastic) may require enterotomy and retrieval of the foreign material (Figs 15.92–15.95) Intussusceptions and prolapsed rectal intussusceptions may be secondary to impactions, foreign bodies, heavy parasitism or neoplasia of the digestive tract.

Electrolyte levels (especially ionised calcium), blood glucose levels and the hydration status of animals suspected of intestinal obstruction should all be examined, and any significant abnormalities should be stabilised prior to any form of aggressive therapy. Long term anorexia and intestinal compromise appear to be better tolerated in chelonians than in mammals. If the condition of the animal fails to improve, these markers can assist the decision either to operate or to continue stabilisation.

Table 15.21 Gastrointestinal foreign body removal.

(1) The visceral organs are identified following incision of the coelomic membrane.
(2) The foreign body, impaction or other obstruction should be located and examined. The stomach can only be partly exteriorised through a plastronotomy. The small intestines are relatively hard to identify and exteriorise due to complex mesenteric arrangements. The large intestine can be exteriorised to a much greater extent, but only in certain sections (Fig. 15.98).
(3) Impacted material can be broken up during the coeliotomy by gentle digital massage/palpation of the intestine in combination with a second worker working up the cloaca from outside and irrigating. Alternatively the intestines can be surgically opened and material evacuated if massage is unproductive.
(4) The coelomic cavity is packed off prior to opening the digestive tract, in order to reduce any contamination (Figs 15.92–15.93).
(5) The organ to undergo surgery can be supported by the use of stay sutures or bowel clamps (Figs 15.93–15.94).
(6) Surgical instruments in contact with the contents of the digestive tract must be considered dirty and should be kept separate from clean instruments. It may be advisable to start with a fresh kit when the digestive tract is closed.
(7) The digestive tract is closed using a two-layered closure (PDS, Ethicon). Everting sutures (Fig. 15.95) are prone to leakage if unprotected, but they can help to contain contents of the digestive tract and can be oversewn with further layers of fine-gauge continuous sutures. Other authors prefer single interrupted sutures oversewn with a continuous inverting suture.

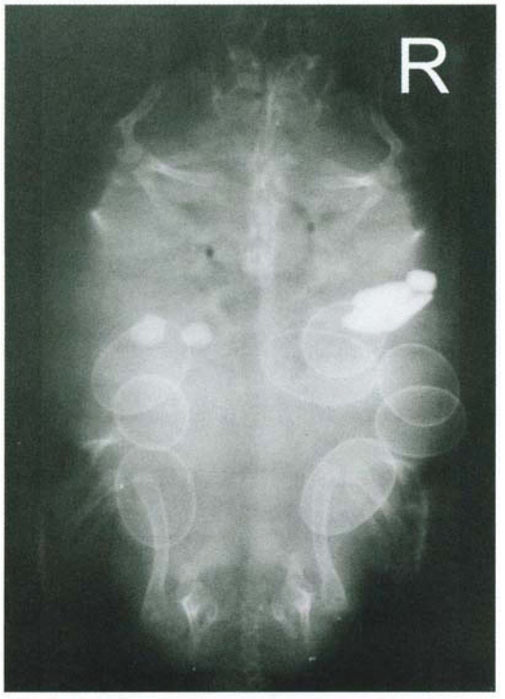


Fig. 15.89 Dorsoventral survey radiograph of a gravid red-eared slider (*Trachemys scripta elegans*) demonstrating significant metabolic bone disease and the presence of eight modestly-calcified eggs.

This animal needs to be treated for nutritional hyperparathyroidism which has been exacerbated by increased calcium demands following ovulation.

Several stones are apparent within the digestive tract. These are likely to be in the stomach and/or large intestine. In this case they are incidental findings but they do suggest an inappropriate substrate and that the animal may have been inadequately fed.

Fig. 15.90 Dorsoventral radiograph of a North American box turtle with an obvious radio-opaque obstruction of the large intestine as a consequence of ingesting large amounts of yellow sand substrate in its enclosure.

This type of impaction generally resolves with improvements in hydration status, cloacal irrigation, lavage and lubrication and oral laxative administration.

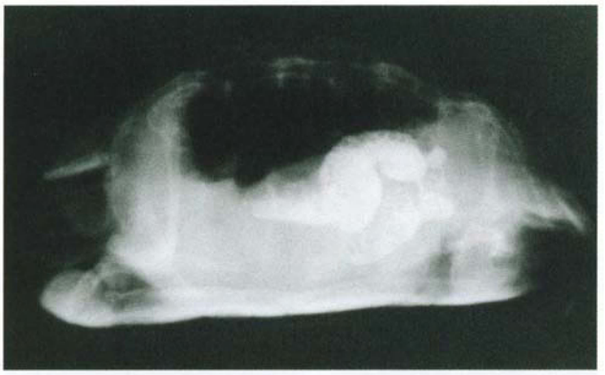
The substrate should be changed to something more suitable. Surgery is rarely required.

Gastrointestinal foreign-body removal

Endoscopic removal of oesophageal and gastric material is difficult but possible given the right equipment (Figs. 15.96–15.97) Endoscopy has been of great help in the removal of plastic bags from the oesophagus of marine chelonians, with the operator reaching down the oesophagus of the anaesthetised animal. Retrieval of foreign bodies through the inguinal soft tissue approach is mentioned elsewhere in this book, but the technique is limited in small terrestrial species, where plastron osteotomy will be required (Bradley 2000) (Figs 15.92–15.95 & 15.98–15.99).

The patient is assessed and then stabilised as described elsewhere. Pre-operative fluids, antibiotics and analgesics are administered as appropriate. A combination of parenteral metronidazole and ceftazidime is recommended. Where surgery is elective and there is the time to do so, the use of oral antibiotics should be considered in order to reduce bacterial load. The aminoglycosides neomycin or paromomycin will achieve this and are minimally absorbed.

A central plastronotomy is performed (see page 416–427). Table 15.21 describes the enterotomy technique.



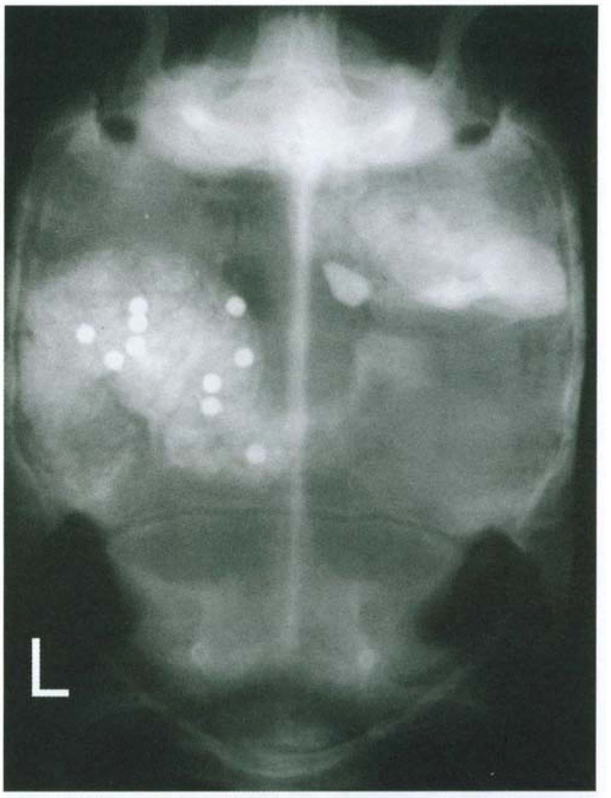


Fig. 15.91a Lateral radiograph of a leopard tortoise (*Geochelone pardalis*) with an obvious obstruction of its large intestine resulting from substrate ingestion. Here the obstruction has been revealed using a mixture of a human water-soluble lubricant jelly and Iohexol (Omnipaque®, Nycomed) in a contrast radiographic study.

This type of impaction is likely to resolve with treatment as Fig. 15.90.

Fig. 15.91b *Testudo ibera:* Intestinal obstruction caused by a gastric neoplasia. There was a secondary gastroduodenal intussusception. This animal is undergoing a contrast study using BIPS.

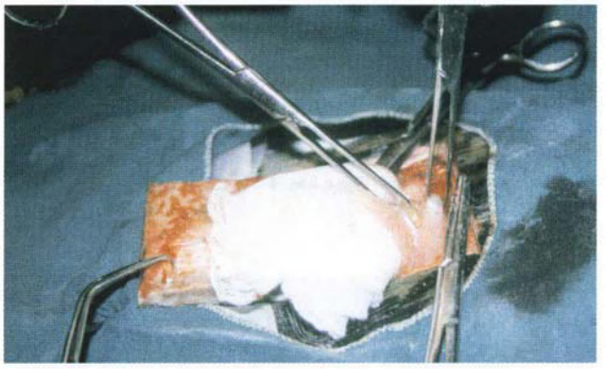


Fig. 15.92 Following plastron osteotomy, the visceral organs are identified. The obstructed digestive tract is exteriorised. The coelomic cavity is packed off prior to opening the digestive tract in order to reduce contamination.

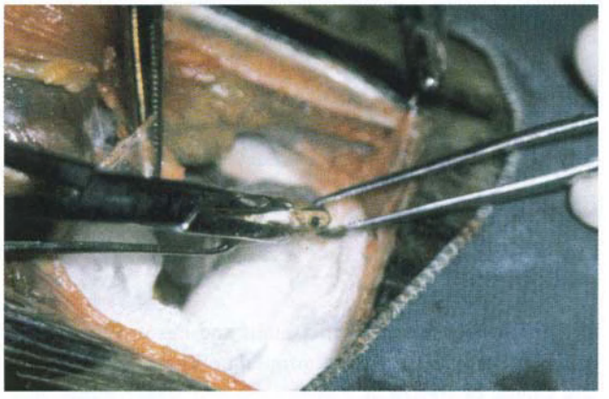


Fig. 15.93 Stay sutures and forceps can be used to stabilise and exteriorise intestines, allowing incision and location of foreign material.

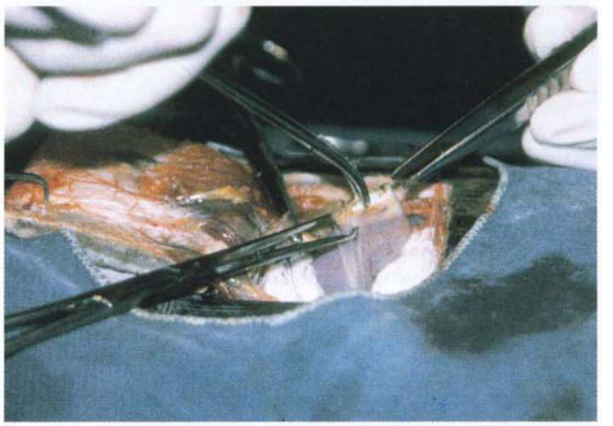


Fig. 15.94 Any contaminated instruments must be discarded and great care must be taken to limit contamination of the coelom with contents of the digestive tract.

TRAUMA

Chelonian traumatic injuries are usually apparent during physical examination, but radiographs should be taken to look for further bony injuries and to assess the nature of the injury. The general health of the animal prior to any injury, and concurrent disease that may affect the healing of an injury, will also need careful assessment. Reptiles in pain will benefit from analgesia and possibly even anaesthesia prior to handling. Haemorrhage and fluid loss should be assessed and the patient stabilised before significant surgical intervention is considered.

Terrestrial chelonians may incur traumatic shell injuries as a result of being dropped by handlers, run over by cars and

Fig. 15.95 Here the digestive tract is closed to produce an initial seal retaining digestive tract material and preventing unwanted spillage. This area is then oversewn with a continuous suture to produce a watertight seal. Copious lavage of the coelom is advisable.

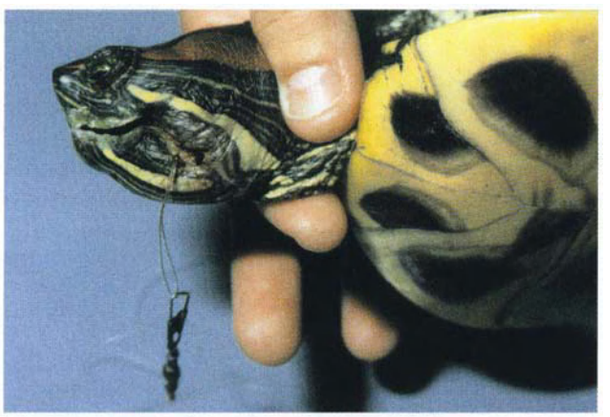


Fig. 15.96 Red-eared slider (*Trachemys scripta*) with a severe injury of the mouth due to having ingested fishing line. On examination it was not obvious if a hook had been ingested or the line was acting as a linear foreign body.
(Courtesy of Jean Meyer)

lawnmowers, gnawed by rats, dogs and other predators, or they may merely suffer a significant fall. Intervention following iatrogenic shell trauma is occasionally required to resolve complications arising as a result of plastron osteotomy. Terrestrial chelonians often cope surprisingly well with carapace trauma and it is common to encounter animals with chronic healed shell deformities resulting from previous traumatic episodes. Osteomyelitis is a common sequel to untreated or inadequately treated shell injuries.

Trauma to chelonian limbs is relatively uncommon, as the their shells give them the ability to withdraw and protect them, but limbs are occasionally presented with fractures and dislocations. Many of these are related to inappropriate handling and may be related to an underlying nutritional osteodystrophy or infection. Fractures are likely in chelonians with nutritional osteodystrophy and commonly occur at the point where a limb is in contact with the shell and subject to a levering force (Fig. 15.100). Animals with presumed limb trauma should be carefully assessed for the presence of metabolic bone disease or articular infections.

Fig. 15.97 Radiography of the red-eared slider in Fig. 15.96 revealed a fish hook within the digestive tract. The hook was located and retrieved using endoscopy. Occasionally such hooks may migrate through the digestive tract and require complex surgical removal if persistent pain or infection results. See also Figs 8.53a–8.55.
(Courtesy of Jean Meyer)

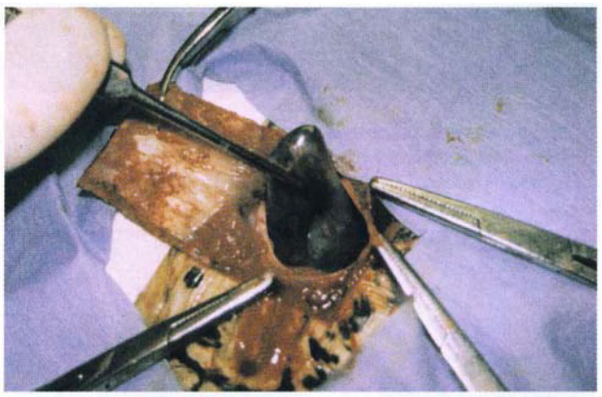


Fig. 15.98 Impactions of the large intestine are relatively easily exteriorised. Here the sheer volume of material within this animal's large intestine necessitates removal.

Clinicians are reminded that limbs and ligaments are easily damaged during handling and surgical positioning, and chelonians should be treated as though they have fragile limbs. Stifle dislocation and cruciate rupture may occur (Divers & Rayment 1999), particularly if the animal is supported by a single hind

Fig. 15.99 Over 100 stones of this size (about 20 typical examples are shown) were removed from a 400 g juvenile leopard tortoise (*Geochelone pardalis*).

The animal had become immobile, anorexic, weak and dyspnoeic, but recovered quickly following correction of fluid and electrolyte imbalances.

Fig. 15.100 Fractures occur at points where limbs may be inadvertently levered on the carapace or plastron, such as has occurred here with this proximal tibia and fibula fracture in a *Testudo graeca*.

limb. Tape the limbs of anaesthetised chelonians in flexion, in order to reduce those forces acting on unobserved limbs during handling (Fig. 14.13).

Inshore motorboats are renowned for their unhappy collisions with surface-swimming aquatic chelonians. Debilitated chelonians,

Fig. 15.101 Turtle head, trauma such as illustrated in this mature loggerhead turtle (*Caretta caretta*) can be the result of boat strikes or trauma inflicted when turtles entangled in fishing lines are released. Animals must be intensively nursed and managed if recovery is to be possible. Nutritional and fluid support, analgesia and antibiotics in conjunction with surgical attention to wounds will be necessary.

Fig. 15.102 This turtle has undergone a hind-flipper amputation as result of entanglement with long fishing line. It has made an excellent recovery and is being assessed in a communal swimming enclosure for possible release.

drifting with currents, or others basking near the water surface, close to the shore, are typical trauma candidates. Circumstantial evidence also suggests that occasionally turtles discovered entangled in float lines of lobster pots and other fishing devices may have been clubbed on the head when freed from entanglement by fishermen (Figs 15.101–15.104). Similarly, turtles may experience limb trauma from entanglements or from inappropriate lifting by flippers during entanglement release. Female chelonians occasionally damage themselves when they collide with beach furniture where a turtle beach is inadequately managed during the nesting season.

SHELL TRAUMA

Because they lack a muscular diaphragm and depend instead on limb movements, chelonian breathing can normally continue despite extensive compromise of the carapace (Figs 15.105–15.106).

Fig. 15.103 Carapace and head trauma in this juvenile green sea turtle (*Chelonia mydas*) is the result of a high-speed motorboat impact in shallow water adjacent to a holiday resort. This turtle had probably been basking when the accident occurred. It was found weak, debilitated and drifting.

Fig. 15.104 Initial stabilisation of cases like this should involve blood glucose measurement and possibly supplementation, epicoelomic fluids, analgesia and antibiotic cover. This animal is easily immobilised by placing it upon blocks and then cleaning its injuries with saline lavage and povidone-iodine solution. The turtle was then dry-docked during its initial rehabilitation.

Fig. 15.105 The posterior portion of the plastron has multiple fractures but the hatchling was still able to walk relatively normally. With supportive care, fluid management, nutritional support, analgesia and antibiotics as described in the accompanying surgical management protocol, this hatchling was nursed to recovery.

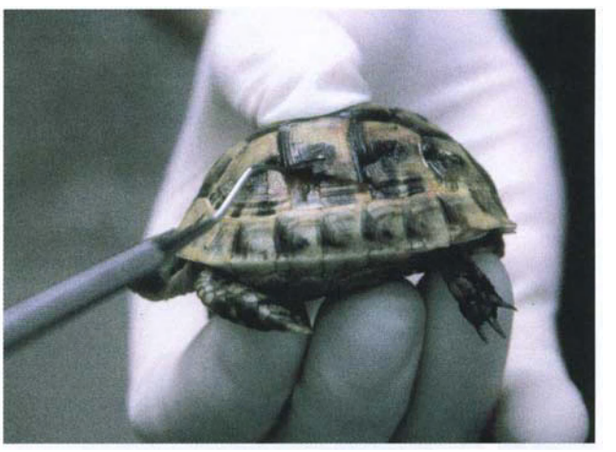


Fig. 15.106 This hatchling *Testudo* has suffered extensive carapacial damage. Lung tissue is visible. Puncture of the carapacial vault does not compromise respiration in the same manner as it would in a mammal. Ventilation in chelonians is not dependent on negative pressure being created by a diaphragm and intercostal muscles. It is instead achieved by altering carapacial volume and tension on the septum horizontale through limb movements.

The spinal cord lies superficially below the carapace in a rudimentary protective bony structure created by the vertebra and connected to the carapace by dorsal spinal processes. Animals experiencing dorsal carapacial trauma or excessive exposure to heat sources, or which have become frozen, may also develop a spinal neuropathy because of associated spinal-cord trauma. A neurological assessment should be performed on all animals with dorsal trauma, and lower-motor-neurone involvement and function should be gauged if possible.

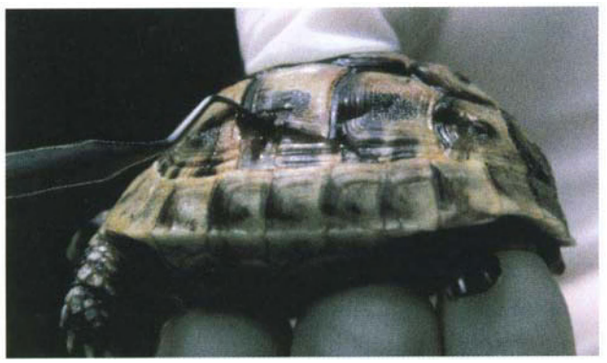


Fig. 15.107 Once stable, and under general anaesthesia, fracture fragments (initially thought missing) are located and reduced. In this case a dental sulcus-cleaning spike is used to reduce the folded fracture.

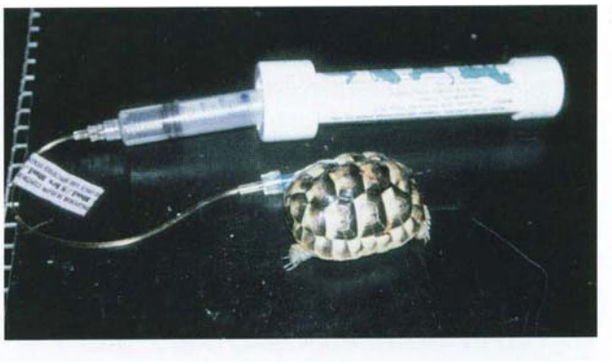


Fig. 15.109 Analgesia, antibiosis and fluid therapy to replace any fluid deficit, such as through this intraosseous drip and Spring syringe driver® (Animalcare, UK), are the initial stabilisation responses to shell trauma in juvenile chelonians resulting from dog bites.

Fig. 15.108 Dog-bite trauma is a common presentation in hatchlings placed outside without adequate protection from other family pets. Often, management of shell damage is a small part of the stabilisation and management of such cases. This animal has been anaesthetised, shell fragments have been removed, fractures have been reduced and an oesophagostomy tube has been placed to help with long-term management. Shell fractures may require very simple medical tape dressings, which are easily changed.

Fig. 15.110 Where the shell has been removed by a dog, but the pleuroperitoneal membrane remains, recovery is normally straightforward and analgesia, antibiotics, fluid and nutritional support and protective dressings are used.

Compromise of the dorsal carapacial vault predisposes the underlying lung and nerve tissue to contamination, inflammation and infection (Figs 15.106–15.107). Ultimately fibrosis, scar tissue, respiratory compromise (and, in the case of swimming chelonians, drowning), are likely consequences. Trauma management should be aimed at reducing further contamination and insult, and allowing the carapace to heal with minimal degenerative change to underlying lung and nerve tissues.

Stabilising and managing acute shell trauma in terrestrial chelonians

Table 15.22 and Figs 15.108–15.113 describe the management of acute shell trauma in terrestrial chelonians.

Dressings

If attended to soon after a traumatic event, defects can sometimes be covered with a semi-permanent material such as epoxy-impregnated fibreglass, but this is unusual. Zeman *et al.* (1967) successfully repaired a mower injury to the carapace of a box

Fig. 15.111 This female *Testudo hermanni* has been extensively gnawed by the family pet dog. Infection was extensive prior to presentation. There are several fistula tracts between the pelvic musculature and the carapace. The keeper of this tortoise must decide between a lengthy course of treatment or euthanasia.

Table 15.22 Stabilising and managing acute shell trauma (modification of advice from Rosskopf & Woerpel 1981).

1. Initially assess and consider options for immediate euthanasia or stabilisation and therapy. Analgesia, antibiosis and fluid therapy, such as an intraosseous drip, may form part of the initial stabilisation process. Wherever possible a patient should be stabilised before any anaesthetic is given.
2. Address haemostasis and hypovolaemia (Fig. 15.109).
3. Flush and clean wounds.
4. Consider anaesthesia to reduce any shell fractures (Figs 15.105–15.107). Avascular and heavily-contaminated fragments of carapace or plastron are best discarded from the outset. Respiration is normally possible without carapacial integrity.
5. Protect lesions of injured animals from fly strike and wound contamination from soil, infected substrate and water in soaks or baths. Consider appropriate dressings (Figs 15.108 & 15.113).
6. Consider simultaneous placement of an oesophagostomy tube during any required surgery to facilitate post-operative management (Fig. 15.108).
7. Check and monitor animals intensively for the initial stabilisation period; consider hospitalisation. Large wounds will cause significantly increased fluid loss. Granulating wounds (such as burns) in other species are colonised in the initial few days by gram-positive bacteria and then subsequently by gram-negatives.
8. Repair and reconstruct any retained but poorly-supported material involved in traumatic lesions.
9. Monitor discharged patients regularly on an outpatient basis.

Fig. 15.112 Dog-bite trauma is a common presentation in hatchlings placed outside without adequate protection from other family pets. Where several hatchlings are managed together, often all are injured. The carapace looks relatively unaffected but the plastron has been extensively crushed and fractured by the action of the dog's lower canines (Fig. 15.105).

Fig. 15.113 In cases where juveniles have had moderate carapacial or plastron trauma, dressings attached using simple surgical tape stabilise the fragments. These dressings can be replaced frequently. Anaesthesia will be required to debride tissues and provide lavage, but simple cleaning and wound inspection may be done using analgesics.

turtle using an epoxy resin. Generally, such wounds must be treated in this way very soon after they are inflicted, and prior to significant wound contamination. Mitchell (2002) suggests that primary closure remains possible up to six hours post trauma. It is possible to apply mesh, material or cloth bandages (e.g. NuGauze® or Bioclusive®, Johnson and Johnson; Tegaderm®, 3M Healthcare) instead of sheets of fibreglass, and to cover these with an epoxy-resin. Such dressings will help to stabilise fragments, may still facilitate drainage, and are occasionally suited to regular changing. However, most wounds should be considered contaminated, and therefore not amenable to primary closure.

When managing traumatic injuries, great care must be taken not to seal an infected site, as chronic infections are likely to overwhelm both the site and the patient. Drainage and regular cleaning of the contaminated trauma site are essential in the immediate post-trauma management period in order to eliminate bacterial and fungal opportunist infections. It is likely to take several months for a non-surgically-created wound to become stable enough and sufficiently free of contaminants to bury below fibreglass or another semi-permanent cover. Delayed closure after the management of any initial contamination, or simple uncovered healing by second intention, are generally highly effective in most trauma cases. This author (SM) would generally manage contaminated shell wounds with lavage, analgesia, local and systemic antimicrobial therapy, bandages, tapes and Elastoplast, or even metal pins, staples and wires (see Orthopaedic Fixation, below).

Most traumatic shell defects are best protected from further contamination and insult with dressings that allow regular changes, for up to three months. This author uses dilute povidone-iodine to clean and flush injuries and wet-to-dry dressings to protect them: IntraSite Gel® (Smith and Nephew), a dry dressing (Rondopad®, DEWE+Co), and, later, perforated radiography film.

New bone generally bridges shell defects after one to two years. Where the shell has been removed but the pleural membrane remains, recovery is normally straightforward. Protective dressings, pain relief, antibiotic cover and general nursing may be all that is required.

Extensive or chronic presentations may require a long and potentially expensive course of treatment. It may be necessary to

consider euthanasia of severely-affected individuals. However even extensive trauma can be adequately stabilised and allowed to heal over time.

Osteomyelitis and neoplasia

In Figs 15.142–15.145, surgical debridement of a chronic carapace dog-bite injury is illustrated. This case demonstrates the extent to which osteomyelitis and abscess/fibriscess formation may occur in the absence of significant external signs. It is important that necrotic and degenerate infected material is removed as part of the successful management of such injuries.

Following debridement, open wounds and shell lesions are best managed conservatively using bandages and dressings. A similar case involving surgical exposure and drainage of a plastron abscess/fibriscess is described by Lawton (1996). Cooper *et al.* (1983) describe the removal of a neoplastic soft-tissue mass invading the site of a previously-surgically-debrided area of osteomyelitis of the anterior plastron of a *Testudo hermanni*. The soft-tissue mass was later identified histopathologically as a neurilemmal sarcoma.

Orthopaedic fixation

Where the shell is fractured but there are no significant defects and minimal contamination, reconstruction using screws and tension bands may be possible. Such cases require meticulous flushing and copious wound lavage, and should be sealed only after all foreign material has been removed. Care should be taken to avoid trauma to visceral structures, such as the heart, when drilling through the shell. It is crucial that natural hinges are not immobilised, but conversely it is important to immobilise adequately those areas where muscular attachments of pectoral and pelvic girdles are likely to displace bone fragments.

In the majority of cases fixed with wires, screws or plates, the fracture site is immediately stable, and the tortoise able to make an uneventful recovery. Care should be taken to check sites for infection, and to ensure that metalwork does not become covered with food debris, faeces or substrate.

To make a screw and wire-tension-band repair:

- Under general anaesthesia, screws are placed at right angles to the shell, either side of the fracture. This may involve measurement of hole depth, and tapping the screw thread.
- Orthopaedic wire is then connected between these screws in an AO tension-band loop. This is used to apply compression to the fracture site.

Screws placed by the authors have been removed after approximately 12 months, with excellent results. The use of screws and tension bands is illustrated. Following fracture healing and removing of the screws, the remaining holes can be covered with protective tape (e.g. Durapore®, 3M). This is changed regularly until healing has occurred, which may take around six weeks (Figs 15.114–15.125).

This technique can only be used confidently in situations where contamination and infection of the injury have been prevented and the wound attended to rapidly. If wound contamination and infection are likely to be established, stabilisation with dressings and bandages, culture and sensitivity analysis of microbial invaders, and wound debridement and lavage should be

Fig. 15.114 This mature female *Testudo graeca* was driven over by the family in a Volkswagen.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

Fig. 15.115 The referring veterinarian provided analgesia, wound lavage, epicoelomic fluids, wound protection and antibiotic cover.
Antibiotic powders should not be introduced into crevices because they interfere with primary osteogenesis.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

Fig. 15.116 After initial assessment by the author (SM), the tortoise was anaesthetised using alphaxalone/alphadolone (Saffan®, Schering-Plough Animal Health) and the carapacial fracture was cleaned and then reduced.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

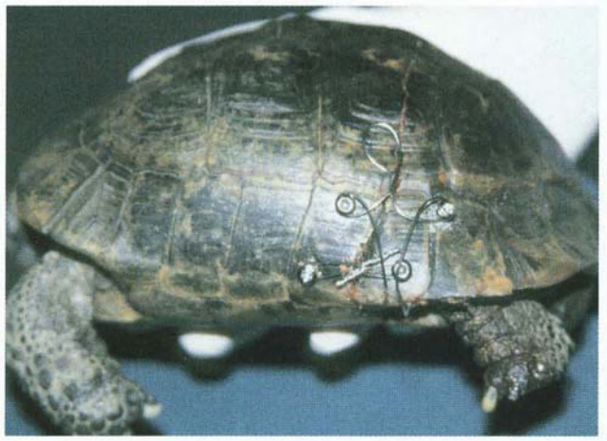


Fig. 15.117 ASIF screws (Animalcare®, York, UK) and tension bands of orthopaedic wire provide simple but effective compression across the fracture site.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

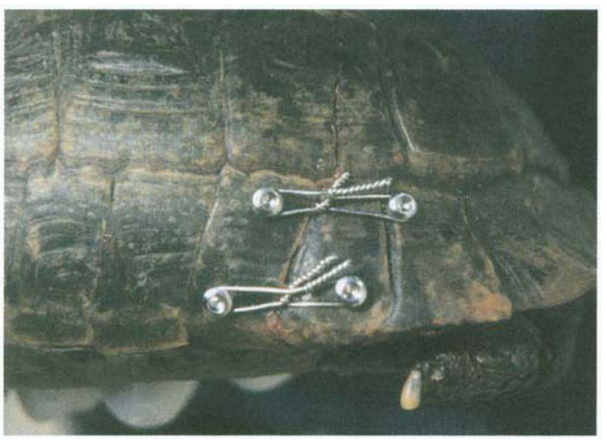


Fig. 15.118 Within two weeks this tortoise was relatively unaffected by its problem. Management at this time was on an outpatient basis.

Fig. 15.119 The anterior gular section of the plastron of this mature female *Testudo graeca* has a hinged and non-displaced fracture. The muscles and visceral structures on the medial side of the fracture have prevented excessive displacement.

Fig. 15.120 Screw holes are drilled across the fracture line, which is reduced and stabilised with bone fragment holding forceps. Care is taken to limit the depth the hole is drilled in order to prevent trauma to underlying structures.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

undertaken. Primary closure should be delayed until the infection is considered controlled or manageable.

Plastron trauma (burns and infections)

Ventral heat sources are to be avoided wherever possible. Chelonian anatomical conformation is geared up to receive and dissipate dorsal radiant heat and not ventral heat. This concept has already been discussed in the Physiology section of this book. Many heat pads develop unsuitable hot spots as they age, and must be checked regularly, and discarded if faulty (Figs 15.126–15.131). Should ventral heat pads be used, it is better to place them on the sidewalls of enclosures, to avoid the possibility of a direct burn.

Excessive ventral heat from a non-thermostatically-controlled heat pad, or a heat pad that has become unreliable and patchy in its output, may create dramatic burn injuries to the plastron and ventral limbs, and result in vast areas of shell necrosis. In very small animals of 100 g or less presented to this author (SM), the effect of direct heat from below on the digestive tract occasionally leads to excessive fermentation, intestinal rupture and a necrotising coelomitis. This occasionally bursts through the plastron, following the digestive tract, to discharge through fistulas. Treating such animals is not realistic and euthanasia is generally advisable.

Immobile animals placed upon ventral heat sources often experience serious injury to the plastron as a result of faecal and

Fig. 15.121 Hole depth is measured allowing the selection of an appropriate screw length.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

Fig. 15.122 Holes are then tapped. The tap is not allowed to penetrate through plastron and damage underlying structures.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

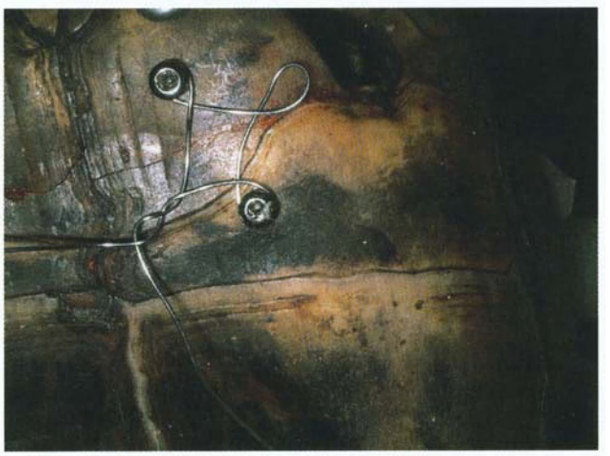


Fig. 15.123 The use of an AO loop tension band made of orthopaedic stainless steel provides compression across the fracture site. The loop is placed around two of the screws either side of the fracture line.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

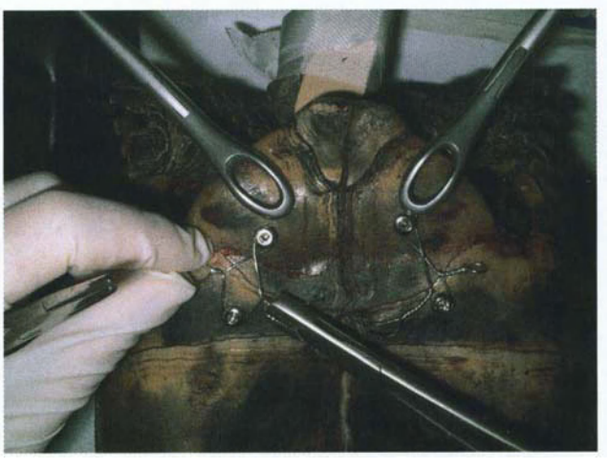


Fig. 15.124 This fracture was immediately stable post-operatively and the tortoise made an uneventful recovery.

Care should be taken to check sites for infection and to ensure that metal work does not become covered with food debris, faeces and substrate. Metal work placed in the plastron will generally require removal after six to eight months if the animal is appropriately maintained.
(Courtesy of the *Journal of Herpetological Medicine and Surgery* and S. McArthur)

urinary contact with the plastron. They may also develop infective lesions of the soft tissues of the proximal limbs (Figs 15.126–15.131). Bacteria are effectively given an ideal environment to invade the animal, because the pad incubates them. Oxygen levels and humidity are also frequently ideal for the bacteria and to the detriment of the patient.

Management

Where animals are presented with ventral trauma, infections, or burns they demand a nursing protocol allowing them to be checked frequently throughout the day, protection from excessive

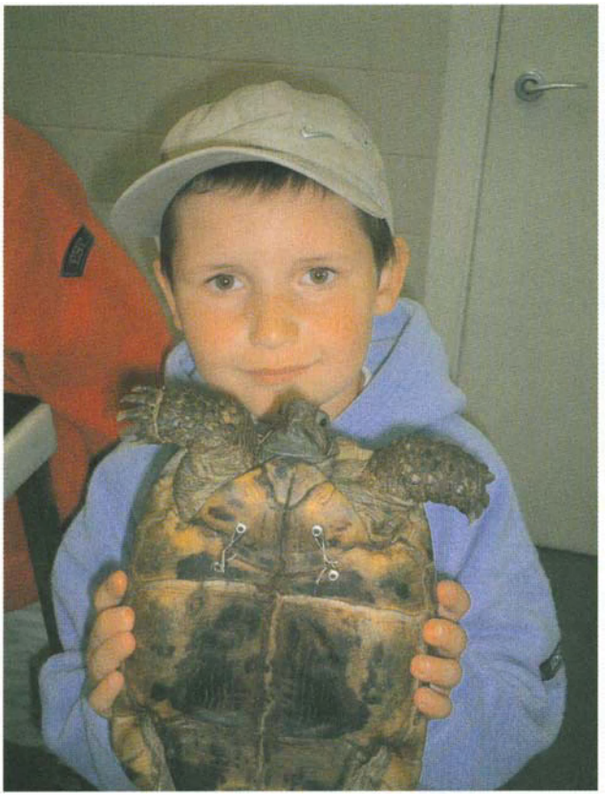


Fig. 15.124a The same animal with its owner a year and a half later. The screws and wires were removed that day and simple tape used to protect the screw holes from soil contamination for the subsequent four to six weeks of healing.

Fig. 15.125a Spade trauma in an Afghan Tortoise (*T horsfieldii*). Twice its owner struck this animal as he dug over his garden in early autumn. The animal had dug itself down into the soil in preparation for hibernation.

exposure to heat and regular cleaning of faecal and urinary contamination. Occasionally barrier creams and dressings will be needed to protect open lesions. This author tends to use slatted trays and draining mats to elevate animals and limit ground contact. This is especially important in species maintained at high humidity, where maximum ventilation of plastron infections and prevention of further faecal contamination is essential.

Fig. 15.125b During an uneventful recovery, an oesophagostomy tube was left in place for two months until the animal was keen to eat on its own once more.

Most traumatic shell defects are best protected with dressings, which allow regular changes for up to 3 months. This author [SM] tends to use dilute povidone iodine to clean and flush injuries and wet to dry dressings [utilizing gel and non-adherent bandages] to protect them.

New bone generally bridges defects after 1–2 years. It is wise to delay primary closure of infected lesions using dressings and therefore not cover them with fiberglass or other semi-permanent material. Where the shell has been removed but the pleuro-peritoneal membrane remains, recovery is normally straightforward. Protective dressings, pain relief, antibiotic cover and general nursing may be all that is required.

Fig. 15.125c Same animal as 15.125a

The first blow skimmed off an area of all hard carapacial structures.

Here the sedated animal has had its wound cleaned of soil and other debris, ready for dressing. Analgesia and antibiotic cover has been provided.

LIMB TRAUMA

Bandages (external coaption)

The short chelonian limbs and their emergence from within the shell make the use of casts and dressings to immobilise limb fractures and traumatic injuries less attractive than with lizards, whose limbs can be taped to the body and tail in positions to reduce fractures. Chelonians present a completely different challenge, because the shell and short tail are not readily available as supports to be incorporated into extension splints.

Fig. 15.125d The area of traumatic carapacial removal was treated with wet to dry dressings as described in the text in relation to dog bite injuries. Here the tissues can be seen to be thickening and developing fibrous tissue 10 days post trauma.

Fig. 15.125e After 6 weeks management, mainly involving application of dry protective dressings as Illustrated above, the carapace has regenerated and a substantial layer of fibrous tissue covers the trauma site.

Fig. 15.125f Same animal as 15.125a
The second blow cut sharply into the carapace with potential to damage both the spine and lung tissues.

Fig. 15.125g The penetration injury was opened up using a burr and Dremmel® drill, allowing removal of soil contamination and inspection of underlying structures.

Despite their shells, though, fractures can be immobilised, and any pain associated with the movement of unstable fractures reduced, by taping either forelimbs or hind limbs in flexion within the shell. Careful radiographic follow-up is important to monitor callus formation and healing. Dressings must be changed regularly if soiled, or where they have slipped and no longer immobilise the fracture in an appropriate position for repair.

External fixation

External fixators are simple and cheap. However, they are not easily applied to chelonian limb extremities alone because they generally foul the shell during limb withdrawal. In such cases it may be best to include an anchorage point to the shell. External fixators can be created using pins, hypodermic needles and thermosetting plastics.

Internal fixation

Mitchell (2002) points out that internal fixation is a surgical option for chelonians with simple, non-comminuted fractures that cannot be stabilised with external support. Alternatively, it may also be appropriate where a reptile is aggressive and difficult to handle, and its behaviour is likely to damage any external support around a repaired fracture. Cerclage wire, intra-medullary pins and plates and screws have all been used to stabilise limb fractures in chelonians, although their use is generally restricted

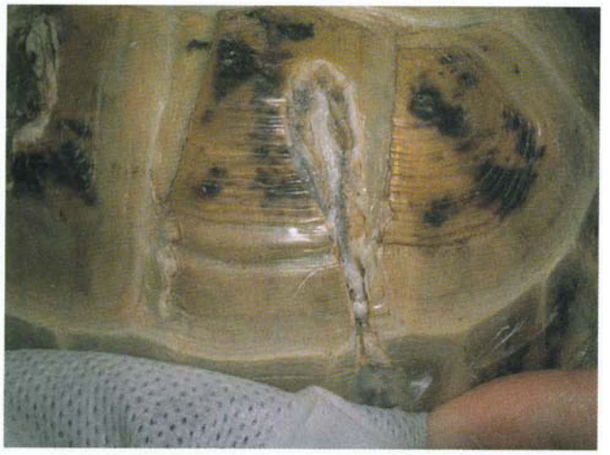


Fig. 15.125h The flank lesion is effectively healed at 6 weeks post trauma. There is already a degree of calcification but most of the tissue filling in the defect is still proteinaceous.

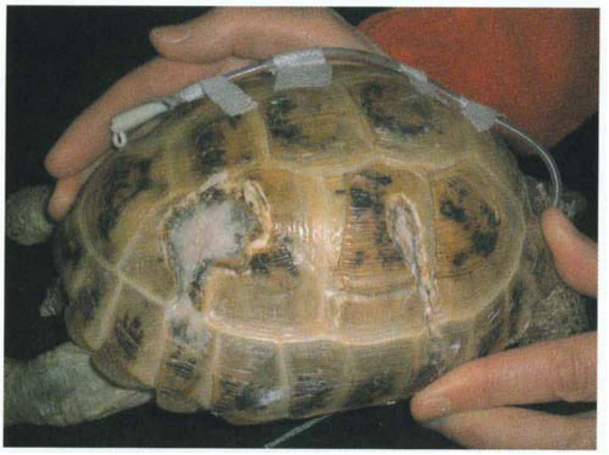


Fig. 15.125i The oesophagostomy tube and dressings can be withdrawn as the animal is able to complete its recovery without further intervention.

to large zoo specimens and those of high financial and conservation worth.

Following surgery, the chelonian will need careful observation for the possibility of a secondary osteomyelitis or implant failure. It is inappropriate to release animals into the wild without first confirming that any implant is free of obvious complications.

Crossed K wires were used to support a distal femoral fracture in *Chrysemys picta* (Mitchell 2002) and the use of a neutralisation bone-plate to repair a humeral fracture in an Aldabran tortoise was described by Crane *et al.* (1980). However, the contours of reptilian bone make plate contour creation complex and unrealistic in many cases. Additionally, the cost and surgical effort involved in implantation make the technique beyond the scope of most chelonian cases.

Ligament repair

Cruciate rupture and stifle disarticulation in terrestrial chelonians

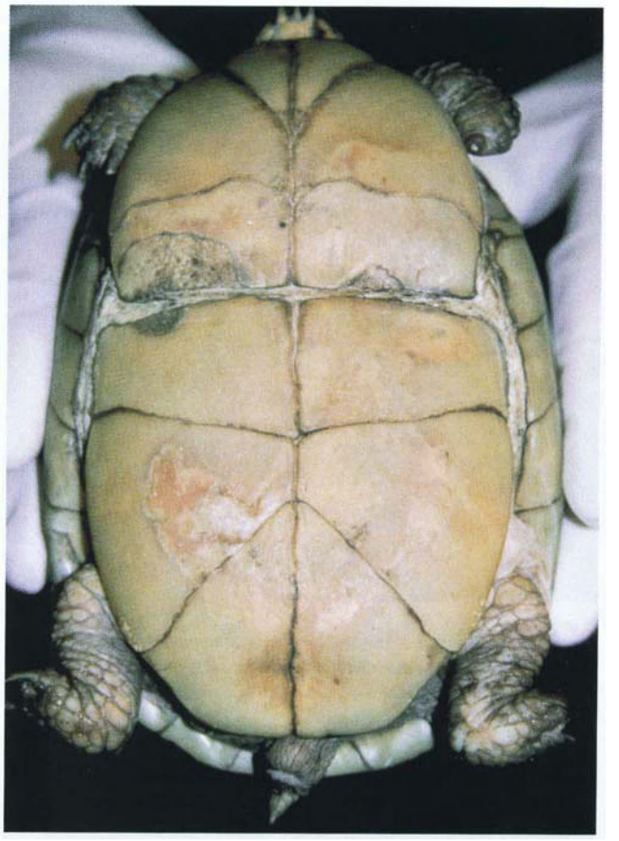


Fig. 15.126 This box turtle (*Terrapene* sp.) was presented with extensive ventral heat trauma to the plastron as a result of housing it in a structure with hot pipes running beneath it.

Fig. 15.127 Extensive separation of the plastron scutes has occurred as a result of bacterial and faecal contamination.

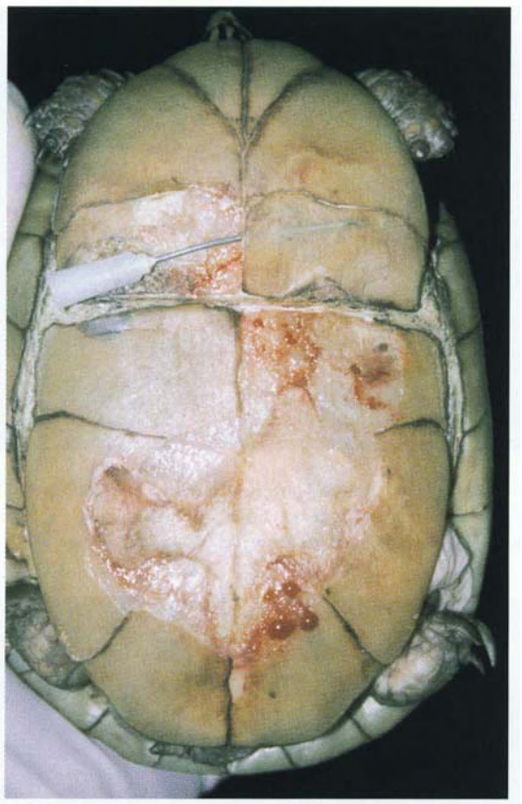


Fig. 15.128 Under general anaesthesia, affected scutes were removed using a hypodermic needle and a scalpel blade. Material was submitted for microbial culture and sensitivity testing and the animal was dry-docked on a slatted draining mat designed for use on a kitchen sink draining board. This helped to minimise ground contact, ventilate the lesion and promote healing in a dry environment during the following weeks.

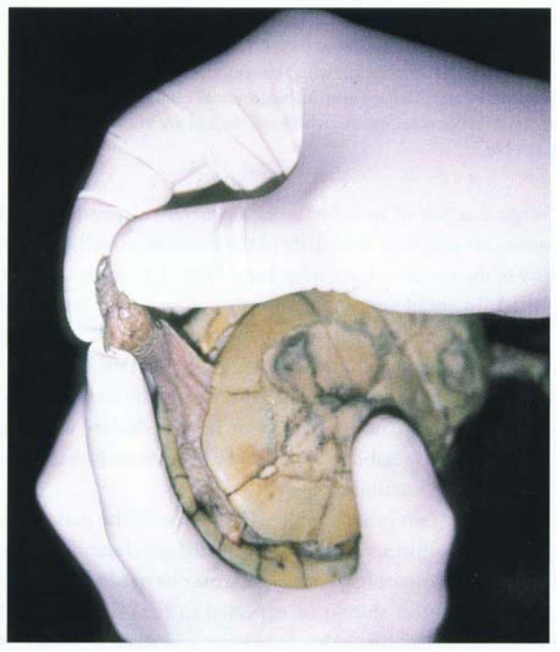


Fig. 15.129 Animals with ventral heat trauma often have lesions on the limbs in addition to plastron damage. This animal made an uneventful recovery in the following three months.

Fig. 15.130 *Geochelone sulcata* with extensive ventral damage as a result of excessive exposure to ventral heat from a poorly- monitored heat pad. Inactive animals may habitually lie upon heat mats. This can result in the incubation of organisms from urine and faecal contamination.

Once osteomyelitis is present, extensive debridement and removal of bone will be necessary to produce a cure. Medical management based upon culture and sensitivity of microbial organisms present is likely to be prolonged. Animals can be raised off the ground to allow wound ventilation during healing.

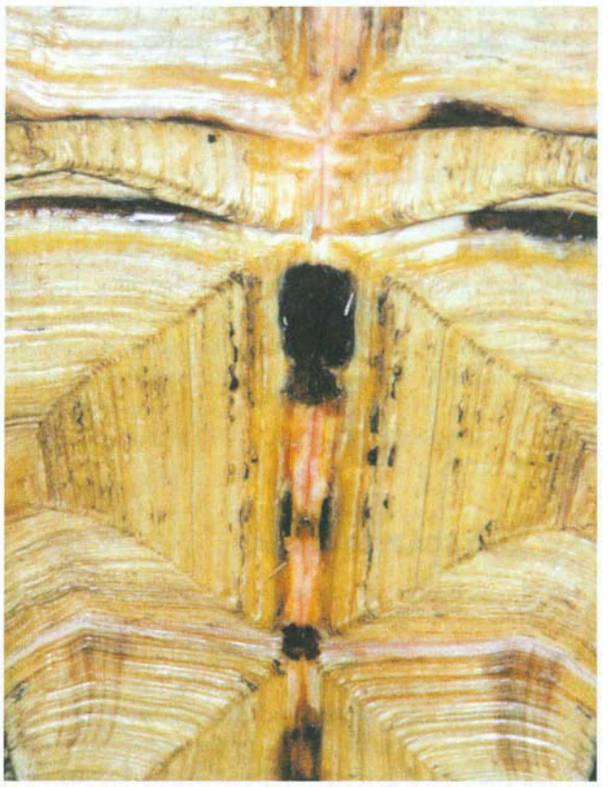


Fig. 15.131 Close up of the same animal as in Fig. 15.130. Four fibreglass pegs were glued to the corners of the plastron. These acted as a supporting trestle, preventing the infected plastron from continued ground contact. The lesions were debrided and antibiotic and analgesic therapy commenced.

may be the result of inappropriate support using the hind limb or of leaning on an upturned chelonian (e.g. during coeliotomy).

A successful technique used to repair this type of injury is described by Divers & Rayment (1999).

Amputation

This may be the treatment of choice for terrestrial chelonians with severe limb trauma or chronic septic arthritis (e.g. with poor response to medical management and PMMA antibiotic bead implantation). Marine chelonians may require flipper removal following chronic entanglement and strangulation in fishing nets and lines.

- High amputations are always indicated to minimise future stump trauma (Bennett 2000).
- Wherever possible, healthy-looking soft tissues should be preserved, to protect and create the stump.
- Limbs can be disarticulated and nerves should be cleanly incised. Bennett (2000) suggests that local application of bupivacaine (max 2 mg/kg) may reduce post-operative pain.
- Skin sutures are generally removed after four to six weeks.
- Lego® wheels, a sectioned billiard ball, a wooden block, a furniture coaster or some other prosthesis can be used to reduce plastron trauma if the animal has difficulty raising its plastron off the ground during locomotion.
- Marine chelonians may require a temporary period of limited water contact while the stump stabilises following surgery.
- The surgical site requires careful monitoring in the immediate post-surgical period for possible infection and wound breakdown.
- Marine chelonians should be given a suitable period to free-swim and rehabilitate under observation in order to check future wild viability. There are ethical concerns when considering the release of amputees into the wild.

RAT-BITE TRAUMA

Hibernating chelonians are regularly prey to trauma from rats entering the hibernaculum. Typically, the flesh of the fore- and hind limbs is stripped away, exposing bone and other deep tissues. Animals should be provided with antibiotic cover and pain relief at the same time as warming and fluid therapy necessary to reverse hibernation. Animals often respond well to treatment but wounds may require long-term management with dressings or grafts to facilitate healing. An example of rat trauma is illustrated in Figs 15.132–15.138.

JAW AND BEAK TRAUMA

Jaw and beak trauma are common in tortoises that have been dropped or hit by cars. Injuries occasionally result from overgrowth of beak tissues (Figs 11.10–11.11). Jaw and beak injuries often render a chelonian unable to prehend, tear or masticate food. If relatively minor injuries, such as fractures of small pieces of keratin, are present, no specific treatment may be needed. For larger keratin defects without loss of bone integrity, small acrylic patches may be affixed to restore symmetry and proper occlusion. Fractures of the maxilla or mandible may need surgical intervention, particularly when fractures are full-thickness and result in significant instability.

Fig. 15.132 This spur-thighed tortoise (*Testudo graeca*) was revived from hibernation when rats were heard within its hibernaculum. The right forelimb had been eaten away leaving just the skin as a sleeve.

Fig. 15.133 All four limbs and the tail exhibited some signs of trauma, although the head and eyes were intact. The anterior and posterior carapace also had obvious gnawing injuries. Lesions arising from rat bites require careful lavage and debridement as rat bites are potential sources of mycobacteriosis, to which chelonians are known to be susceptible.

Surgical repair of such fractures is generally dependent on the location, severity and chronicity of the fracture, as well as the creativity of the surgeon. Generally, some type of wiring technique is required. In small specimens, simple cerclage wire may be the only option. A dental drill may be used in such cases to pre-drill the holes through which the wire will pass. Stainless steel wire of 22G or 25G works well in small specimens. In larger animals, modified external fixation devices or intramedullary wires may be used to provide stabilisation. Acrylic bridges may be used creatively in some situations.

In general, if no other problems are present, the patient may begin eating within several days of repair if good stability is provided. If not, an oesophagostomy tube may be needed.

Serious fractures should be expected to heal slowly over the course of six to eight months. Analgesics and antimicrobials may be needed for particularly extensive or contaminated injuries. With proper treatment, the prognosis with most jaw injuries is generally good.

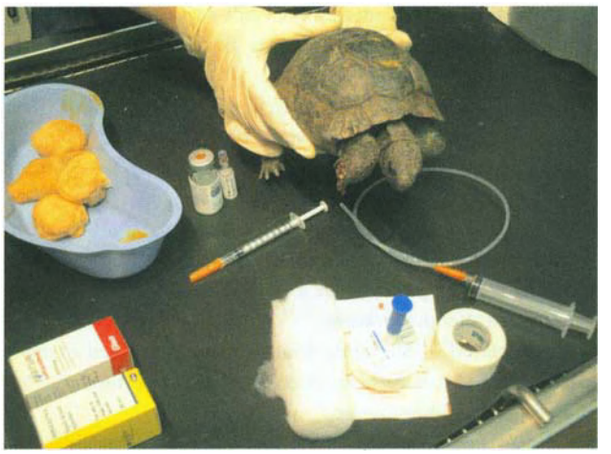


Fig. 15.134 The equipment required to manage rat trauma is fairly basic. Sedation is possible using a combination of midazolam and ketamine or a different agent (refer to the main text for details). Anaesthesia can be achieved through intubation and ventilation using a volatile agent.

Carprofen can be used to provide analgesia and, following harvest of diagnostic samples, antibiotic therapy can be begun using an antibiotic such as ceftazidime. Injuries can be debrided surgically and then packed with a gel such as IntraSite® and then bandaged/dressed. Fluids can be provided epicoelomically and/or via gavage using a syringe and canine urethral catheter.

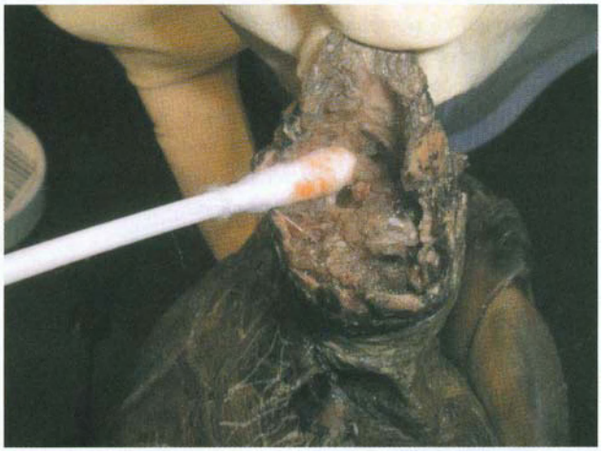


Fig. 15.135 A prudent clinician would take samples from exposed injuries for microbial culture and sensitivity testing. This will help determine the most appropriate post-operative antibiotic.

Mandibular fractures

Lawton (2000b) describes a technique to repair mandible trauma in chelonians. Cases presented with undiagnosed anorexia were found to have unstable injuries of the mandible. Following surgical stabilisation a rapid recovery and return of appetite was recorded, suggesting that unstable fractures of the mandible interfere with prehension and may be significantly painful. Chronic symphyseal fractures may be associated with chronic nutritional osteodystrophy and secondary osteomyelitis and animals should be assessed for further problems. Table 15.23 discusses stabilising mandibular fractures.

Fig. 15.136 Some injuries can be closed down and sutured. Generally, wounds less than six hours old, with limited secondary contamination, are suited to primary closure. If wounds are old or have become necrotic second intention healing is more appropriate. Injuries can be protected with a gel suited to open wound management in combination with a wet-to-dry dressing.

Fig. 15.137 Severely affected limbs are best immobilised by bandaging the limb in flexion using the carapace and plastron to attach surgical tape or other protective material.

Fig. 15.138 One week post-operatively, following hospitalisation and nutritional/fluid support, analgesia, wound management, dressing changes and antibiosis, even extensive trauma may show significant signs of healing.

Table 15.23 Stabilisation of mandibular fractures.

(1) Stabilise the patient, consider underlying disease such as nutritional osteodystrophy and osteomyelitis, and consider biopsy for histopathology and culture and sensitivity.
(2) Anaesthetise and provide appropriate peri-operative fluids and analgesia.
(3) Lawton (2000b) found that mandibular fractures were easiest approached with the animal positioned in dorsal recumbency.
(4) Necrotic material and bone affected with osteomyelitis must be removed.
(5) At this stage placement of an oesophagostomy tube should be considered.
(6) Stabilise fractures with external fixation devices, Technovit® or dental restorative cement and glues. External fixators can be made with hypodermic syringes and Kirschner wires linked with a thermosetting plastic such as Technovit®. Alternatively, a gum shield can be constructed by gluing a thermosetting plastic directly to the beak itself with epoxy resin or glue.
(7) Where pins are used, they are best placed in bone and not just the horn tissue of the beak where they may work loose.
(8) Healing takes around three months and during this time it is essential to check regularly for implant failure and infection.

MARINE CHELONIAN TRAUMA

General advice regarding medical stabilisation and hospitalisation of marine chelonians is given in earlier in this book. Animals should be assessed and stabilised and provided with fluids, analgesia and antibiotic cover following bacterial sampling prior to any surgical intervention. Animals are often debilitated and hypoglycaemic and the reader is referred to the earlier problem-solving approach to turtle disease. Where animals must be nursed out of water or dry-docked for long periods, oesophagostomy tube placement may be helpful in maintaining nutritional input.

Situations where surgical intervention may be required can be broadly categorised into three types, as shown in Table 15.24 below.

In all three cases, it is crucial that animals are stabilised and rehabilitated before being released into the wild. Where facilities to rehabilitate wild turtles are not available, it is important that cases are appropriately assessed at the outset and that euthanasia is considered along with other management options, such as transportation to locations where appropriate facilities are available.

OSTEOMYELITIS

Implantation of antibiotic-impregnated polymethylmethacrylate (PMMA) beads is the preferred treatment for osteomyelitis or septic arthritis (Divers & Lawton 1999). The technique offers the dual advantages of good drug delivery to the infected site and reduced chance of systemic toxicity (Bennett 1999).

- Prior to placement of the antibiotic-impregnated implant it is prudent to biopsy the bone and obtain a microbial culture and sensitivity result.
- At the time of implantation, devitalised tissue should be removed (especially bone). This may involve the use of curettes and burrs.
- Aminoglycosides and clindamycin are the drugs most often used in this way. Divers & Lawton (1999) suggest 2 g neomycin plus 2 g clindamycin per 20 g of PMMA, and reported finding no evidence of nephrotoxicity with this recipe.
- Small beads release an antibiotic quicker than large because of their increased surface area to volume ratio.
- Beads may be prepared in a sterile fashion or may be gas-sterilised after preparation.
- Beads placed within joints should be removed when a cure is considered to have been effected in order to limit further degenerative joint disease.
- Disease progression can be monitored radiographically.

RESPIRATORY TRACT

Some procedures described here fall under the title of diagnostic techniques. However, as they involve procedures that are generally carried out in the anaesthetised or sedated patient, they are included here.

Biopsy of the upper respiratory tract

In cases of chronic upper respiratory tract disease, it is possible to harvest biopsy samples endoscopically using a retrograde technique through an oesophagostomy incision. The endoscope is passed into the oesophagus through an oesophagostomy incision and then advanced rostrally into the choana and nasal chamber (Fig. 15.139). Material can be examined by PCR for the presence of herpesvirus and *Mycoplasma agassizii* and material harvested for viral, fungal, mycoplasma, bacterial and mycotic culture, electron microscopy or cytology/histopathology.

Biopsy of the lower respiratory tract

Divers (2000a) describes two approaches to the chelonian lung and advocates endoscopy as an ideal tool for inspection of lung

Fig. 15.139 Retrograde oesophageal endoscopy in *Geochelone pardalis* with chronic upper respiratory tract disease. Endoscopy allows inspection and biopsy of material from the upper respiratory tract.

Table 15.24 Situations requiring surgical intervention.

Propeller injuries	Propeller injuries range from mild to severe head and eye lacerations to limb trauma and carapacial fractures. • Often chronic lesions may appear healed but in fact deep necrotic areas of tissue may be present and bone fragments and foreign material present are best identified and removed. • Where apparently healed lesions are present on debilitated animals it is important that these are investigated using techniques such as cytology of needle aspirates, radiography and ultrasonography of lesions, and possibly exploratory surgery. • Severely damaged limbs may require amputation. • Microbial culture and sensitivity should be undertaken where possible prior to the commencement of antibiotic therapy. • Fresh wounds should be debrided, debris removed and residual tissues flushed with copious sterile saline. • Tristan & Mader (2000) describe the management of serious head trauma in an 80 kg adult green sea turtle (*Chelonia mydas*). Despite enucleation of one eye and visual impairment in the other, the animal was aggressively nursed and showed a positive response to care over a three-month period in which it began to eat voluntarily. A similar injury in a loggerhead turtle found floating off the coast of Cyprus is illustrated in Fig. 15.101.
Boat strikes	Boats generally strike the head and carapace of debilitated, surface-swimming or floating chelonians (Figs 15.103–15.104). Motorboats often strike animals basking in shallow warm water. • Injuries to the carapace may result in head trauma, spinal trauma and hind limb paresis, or paralysis. • Osteomyelitis and chronic granulomatous infections are common sequels to untreated carapacial trauma. • Long-term management may involve extensive debridement and protection that can be regularly removed to allow wound debridement and lavage. • Marine turtles may be best removed from water and dry-docked during the management of such lesions. • Shell injuries may take two to six weeks for the full extent of all damaged tissue to become obvious (Walsh 1999). • Where spinal trauma results in long-term paresis or paralysis, the animal may have diving weights applied in order to produce stability during shallow swimming. Appropriately-weighted turtles at Marathon turtle hospital have been remarkably adept at swimming and eating. Constipation and colitis are possible long-term complications and such animals cannot be released. • Walsh (1999) advocates the use of Tegaderm® (3M Healthcare) acrylic over surgically-debrided carapacial injuries, with covering dressings changed regularly to allow wound lavage and management.
Entanglements	Entanglements may result in deep lacerations to limbs, soft-tissue injuries and vascular compromise. • Wounds will need extensive surgical debridement; necrotic bone and foreign material should be removed. • Infections should be investigated for microbial culture and sensitivity patterns and wound resection and primary closure attempted where possible. • Uncontaminated wounds closed by primary intention can be immersed in water relatively soon after surgery. • Open wounds may benefit from nursing out of water with protective barrier ointments and systemic antibiotics, fluids and analgesics. • Severe limb injuries may require amputation (Fig. 15.102).

tissue and harvest of material for biopsy and microbial culture and sensitivity testing.

Carapacial access

Pre-operative, horizontal beam, AP and lateral radiographs are taken, to determine the site for the osteotomy. For general access, the site would be centrally in the lateral carapace where the spine can be easily avoided.

Under general anaesthesia, a small drill is used to create the osteotomy. The diameter of the drill is determined by the size of endoscope or other surgical instrument required to pass through the opening. The carapace is drilled with the animal maintained in expiration to avoid excessive trauma to lung tissue.

The osteotomy can be covered with protective tape and will heal in two to four months.

Prefemoral access

This procedure is suited to unilateral caudal lung lesions.

The site of inspection and access is determined radiographically as above. After appropriate aseptic preparation, a small stab incision is made in the craniodorsal aspect of the prefemoral fossa on the side where lesions have already been identified.

The septum horizontale is identified and stabilised with surgical implements or stay sutures. A small incision is made through it and an endoscope is used to inspect lung tissue and harvest appropriate diagnostic material.

Divers (2000a) advocates closure of the septum horizontale with sutures in order to reduce the possibility of post-operative pneumocoelom.

Lung wash

Diagnostic material can be collected for cytology, microbial culture, viral PCR, electron microscopy or pathogen isolation by flushing and then aspirating a small amount of saline through a catheter passed down the trachea of an anaesthetised animal. Murray (1996) suggests 0.5%–1% of the animal's body weight. The catheter can be directed into either lung field with the aid of an angled stylet. Drury *et al.* (1999a) were able to identify viral agents using material harvested endoscopically by this technique (Figs 15.140–15.141).

Fig. 15.140 Lung wash in *Testudo hermanni*. Here a Jackson cat catheter is passed down the trachea and saline used to flush and retrieve diagnostic material. The sample is less likely to be contaminated with organisms from the pharynx if passed through a sterile endotracheal tube.

Fig. 15.141 Lung wash in *Geochelone pardalis*. Material harvested is potentially suitable for virus isolation, electron microscopy, viral PCR, histopathology, cytology and microbial culture.

Lung abscesses

This author (SM) has encountered various infections wherein an entire lung is unilaterally consolidated and replaced with caseous material. In these situations it is possible to curette, debride and remove material through a carapacial osteotomy similar to that described by Divers (2000a) (Figs 15.142–15.147). The osteotomy can be combined with a cranial, prescapular, soft-tissue approach, or a caudal, prefemoral, soft-tissue approach, or both, depending upon the extent of the infection and solid material present.

Invariably, a large amount of abnormal lung tissue must also be removed. If bronchial vessels are adequately ligated, this is a relatively straightforward procedure. Infected solid lung material generally shells away from the pleurocoelomic membrane beneath the carapace. Such surgery is highly invasive and comes with a high degree of risk to the animal, but occasionally allows remarkable resolution. In many cases respiratory distress is immediately removed by allowing increased inflation and use of the remaining normal lung. Alternative treatment is palliative management with antimicrobial cover based upon culture and sensitivity results.

Fig. 15.142 This animal had severe unilateral lung consolidation on craniocaudal and lateral radiography. Externally a small discharging fistula was present.

Fig. 15.143 On closer examination, under general anaesthesia, it was apparent that infection of the underlying lung tissue was extensive (see also Fig. 15.146).

Fig. 15.144 After infected bone and soft tissue were removed, it was decided that the animal was too extensively infected to treat and euthanasia was performed.

EYE ENUCLEATION

Chronic intra-ocular infections or ruptured traumatised eyes may require surgical removal. Eye enucleation in the green sea turtle is described by Tristan & Mader (2000). Chronic pain is also a valid indication, but as pain is so hard to assess it would be a subjective diagnosis based upon observed blepharospasm and self-trauma to the eye with the associated forelimb.

Table 15.25 describes the procedure.

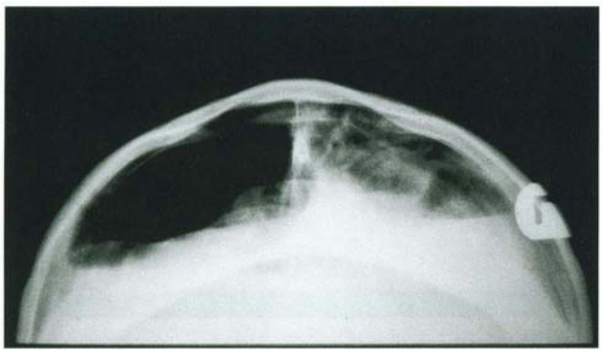


Fig. 15.145 Horizontal beam radiography (craniocaudal) showing left-sided radio-opacity consistent with a unilateral pneumonia or LRTD (lower respiratory tract disease).

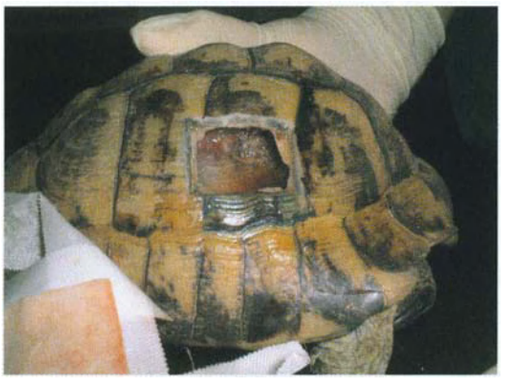


Fig. 15.146 This *Testudo hermanni* had caseous material occupying its entire left lung field. It showed a remarkable improvement in respiratory activity and appetite following unilateral lung removal via a lateral carapacial osteotomy.

Smaller drill holes, made under general anaesthesia, can be used to harvest diagnostic material or apply medication directly into lesions that have been located radiographically. Such access also allows endoscopic inspection and other diagnostic and therapeutic measures.

Fig. 15.147 The osteotomy was successfully managed in a conservative fashion using dressings to prevent wound contamination during the post-operative period.

MICROCHIP INSERTION (TERRESTRIAL AND SEMI-AQUATIC SPECIES)

Under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) legislation, certain chelonian species within the United Kingdom are required by law to be microchipped. It is presumed that this is also the case in many other countries worldwide. Specific conditions relating to the age, size and species of the animal will apply to local CITES legislation and these may even be varied from time to time. The reader is therefore directed towards guidelines issued by the governmental body responsible for CITES enforcement within their own country for specific details.

Table 15.25 Eye enucleation.

(1) The eye is removed along with the lid margins.
(2) There is little free skin, but any defect created can usually be closed if trimming of soft tissues around the eye is minimal.
(3) Any surgical dead space within the orbit is closed down using fine monofilament absorbable sutures (PDSII®, Ethicon).
(4) Subcutaneous sutures can be used to distribute tension evenly and reduce the likelihood of post-surgical seroma formation.
(5) The skin is sutured with sheathed nylon or an absorbable monofilament.
(6) Sutures are generally removed after four to six weeks.

Provided precautions are taken to minimise the risk of sepsis, implantation of a microchip into a tortoise is usually both a safe and effective procedure. Because of the nature of the reptile integument, insertion of a microchip will always pose some risk of abscess formation, even where attention has been paid to sterility. As chelonian skin is inelastic, the insertion site remains open for some time following removal of the needle. Failure to close the insertion site increases the risk of chip loss and infection.

In non-hibernating species, the timing of microchip implantation is not important. However, hibernating species are best implanted in spring, having recovered from their hibernation. Should licensing regulations force implantation close to a hibernation period, it is prudent to keep the animal awake for at least the following six weeks and to monitor the implantation site carefully during this period (Figs 15.148–15.153).

Insertion sites

Two specific insertion sites are described in this text:

- left hind limb;
- midline sub-plastron.

It is anticipated that further sites will become recognised over the next few years and the reader is encouraged to contact the veterinary bodies in their own country for up-to-date advice. In wild marine species the flippers are often used and in private collections of giant chelonian species chips are occasionally inserted into the pectoral muscles or subcutaneously about the neck or the skin of the forelimbs.

Hind limb

Use of the hind limb site poses significant problems in many animals:

- It involves significant handling of the limb which may stress and cause trauma to animals, especially where metabolic bone disease is present. Two people will generally be required to restrain and operate on an animal in order to insert a chip.

Fig. 15.148 Sub-plastron microchip insertion in a semi-aquatic turtle. This site requires limited aftercare and therefore facilitates early return to water. This author (SM) favours a sub-plastron site where chip implantation is required in a chelonian.

The site should be adequately cleaned and prepared.

Restraint and handling required for chip insertion at this site are far less stressful for the animal and operator when compared with insertion in the hind limb site favoured in some protocols. The reader is referred to any protocols issued within their own country, or by governing organisations.

It is prudent to keep animals such as that illustrated out of water for 24 hours. Terrestrial chelonians may require a small amount of tissue glue to close the site. A suture is not generally necessary.

Fig. 15.149 Anatomical location of the sub-plastron implantation site (post-mortem specimen, plastron removed). (Death was not related to chip insertion!).

This area is relatively free of blood vessels, nerves and other structures. Fascial planes reduce the likelihood of chip migration.

It is well tolerated, especially in delicate animals.

Fig. 15.150 Lateral radiograph of a breeding female *Testudo graeca*. A microchip (required by United Kingdom DEFRA regulations) is clearly visible in the tissues dorsal to the hind limb.

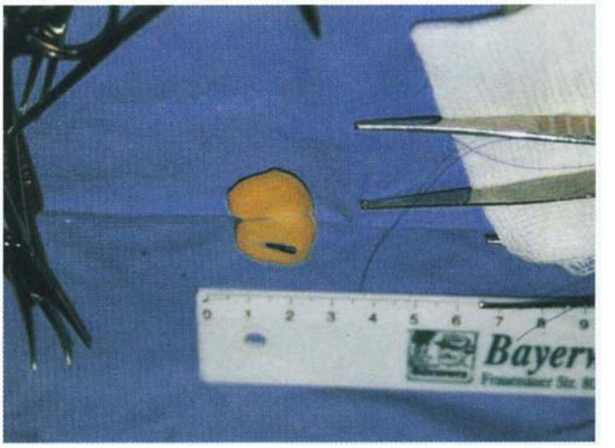


Fig. 15.151 Complications associated with microchip insertion include abscess formation. An abscess may occur despite high levels of sterility sought at the time of implantation.
(Courtesy of *Journal of Small Animal Practice* and Mike Jessop)

Fig. 15.152 Implantation of the microchip in the left hind leg using a single-unit proprietary chip.
(Courtesy of Mike Jessop)

- If the ventral limb surface is used it may affect the femoral vessels and nerves, and if the dorsal limb surface is used, insertion may affect the sciatic nerve and associated vessels.
- The site is difficult to disinfect adequately.
- Box turtle and hingeback species are able to close their femoral fossa completely. Other species may be so large and powerful that sustained extension of the hind limb is impossible. In

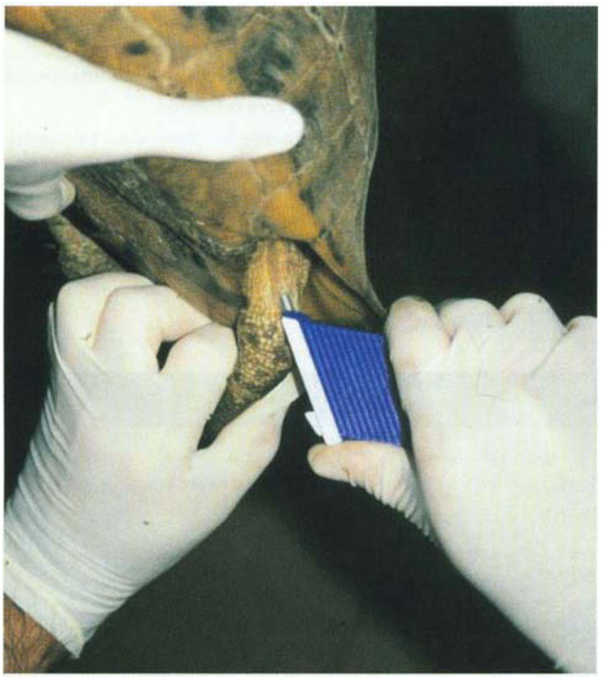


Fig. 15.153 Implantation of a microchip in the left hind leg using a gun and detachable needle.
(Courtesy of Mike Jessop)

such situations sedation may be required. If dealing with a large number of animals, all this will be very time consuming to the handlers and may cause distress to the animals.

- The subcutaneous limb site has a risk of implant failure and migration.

Table 15.26 describes the technique for inserting a microchip in the hind limb.

Midline sub-plastron

An alternative site is in the ventral midline connective tissue between the pubis and the plastron. This can be reached by a needle inserted just ventral to the tail and just above the caudal edge of the plastron. This site has been used regularly by one author (SM) in the United Kingdom as well as in an introduced population of over 400 *Trachemys scripta elegans* on the Eckerd College campus in St. Petersburg, Florida. These animals were monitored without obvious complications for about ten years following chip insertion (Meylan 2002: personal communication). Microchips inserted in this area are still picked up by most standard chip readers placed over the left hind limb.

The sub-plastron site has various differences to the hind limb site, most of which are advantageous:

- Little handling is required and only one person will generally be required to restrain and operate on an animal in order

Table 15.26 Inserting a microchip in the hind limb (modified from Jessop 2000).

Site landmarks	Historically, the internationally agreed site for microchip insertion in chelonians in the United Kingdom has been the subcutis of the dorsal or ventral surface of the left hind limb. The microchip is generally best placed subcutaneously if the animal is large enough. In smaller animals it must occasionally be placed intramuscularly. Care should be taken to avoid the femoral vessels and nerves and the sciatic nerve, kidneys and lung tissue.
Preparation	Faecal material is rinsed off. The site is then surgically disinfected by gentle scrubbing with a toothbrush and iodine scrub soap. The soap can be dry-wiped or rinsed off with a small volume of surgical spirit.
Restraint	Some animals will require sedation; some will require restraint by an assistant with the left hind leg in extension towards the operator.
Analgesia	The implant site is infiltrated with a small volume of local anaesthetic (Anaesthesia chapter). In adults, the skin is thick and intradermal and sub-cutaneous infiltrations are needed to ensure an effective block. If the chip is to be placed intramuscularly, some infiltration into the muscle is required. A temporary functional lameness is possible until the local anaesthetic wears off.
Incision	In older animals it is recommended to incise the skin with a scalpel. Younger animals one can implant simply using the needle supplied with the chip.
Insertion	The animal should be restrained appropriately. Ensure that excessive force is not applied to limbs, which can be vulnerable to ligament and bone damage, particularly where previous care and husbandry have been poor. Position the needle either subcutaneously or intramuscularly and inject the chip.
Haemorrhage	Very occasionally haemorrhage follows needle withdrawal. This is more commonly seen with intramuscular implantation. Haemorrhage is generally easy to control using a sterile cotton bud to apply local pressure.
Closure	A small amount of sterile tissue glue can be used to close the implantation site or a single mattress suture can be used to produce eversion of skin edges. Skin staples are also effective.
Post-insertion	Maintain the animal in a clean environment for a few days afterwards, especially digging species or those allowed to roam free in a garden. Sutures should be left in place for a minimum of four weeks, after which they can be removed. During the following two weeks the area should be checked for evidence of infection. The animal should be scanned a week or so later to ensure the chip has remained in its correct location.
Semi-aquatic species	Semi-aquatic species require extra care after insertion. If possible maintain in a dry environment for two days. Most risk of infection is from contamination via poor water quality.

to implant a chip. It is not necessary to restrain and possibly injure limbs.

- No major vessels and nerves lie within the implantation area.
- The site is still complicated to disinfect adequately.
- Species that are able to close their femoral fossa completely, as well as large, powerful species, allow a microchip to be inserted relatively easily without need for significant restraint or sedation.
- The sub-plastron implantation site is filled with connective tissue and a minimum of muscle. This reduces the possibility of haemorrhage, nerve damage and chip migration compared to a limb site.
- A long-handled insertion gun may be required in some species where access to the subplastral area is limited.

Table 15.27 describes the technique for inserting a microchip in the sub-plastron site.

OTHER SURGICAL PROCEDURES

Table 15.28 lists some miscellaneous surgical procedures.

Table 15.27 Inserting a microchip in the sub-plastron site.

Site landmarks	Implant the microchip between the pubis and the plastron. This is an area that is filled with connective tissue and a minimum of muscle. It is located by inserting the implant needle parallel to the plastron, through a point beneath the tail and cloacal opening following a line in the midline between the two caudal anal scutes.
Preparation	Faecal material is rinsed off. The site is then surgically disinfected by gentle scrubbing with a toothbrush and iodine scrub soap. The soap can be dry-wiped or rinsed off with a small volume of surgical spirit.
Restraint	A single person can usually manage. The animal may be best placed in dorsal recumbency on a support.
Analgesia	The implant site is infiltrated with a small volume of local anaesthetic (see Anaesthesia chapter).
Incision	The insertion needle is generally adequate to puncture the skin.
Insertion	The needle is inserted just ventral to the tail and dorsal to the plastron, entering the skin in the flesh just caudal to the plastron in the midline. The needle is inserted into the connective tissue below the pelvis in the midline and the chip expelled.
Haemorrhage	As no major vessels are found in the implantation site haemorrhage is unusual. If there is any bleeding, it is generally easy to control using a sterile cotton bud to apply local pressure.
Closure	A small amount of tissue glue can be applied to the implantation site to seal the skin. Suture placement is not usually required.
Post-insertion	Maintain in a clean environment for a few days afterwards, especially digging species or those allowed to roam free in a garden. Sutures should be left in place for a minimum of four weeks, after which they can be removed. During the following two weeks the area should be checked for evidence of infection. The animal should be scanned a week or so later to ensure the chip has remained in its correct location.
Aquatic species	Any water-dwelling animal can be maintained out of water in a damp holding container, such as a clean plastic bucket or storage container, on a damp towel for a few hours. Once the glue has set, such an animal can be returned to water once more, or maintained in the damp container depending upon individual circumstances.

Fig. 15.154 Conjunctivitis and the presence of caseous plaques associated with hypovitaminosis A in a red-eared slider *Pseudemys scripta*.

Fig. 15.155 With the animal sedated and local anaesthetic applied to the eye, or alternatively under general anaesthesia, caseous material is gently removed. Fine tissue forceps and dampened cotton buds can be used.

Table 15.28 Some other general surgical procedures.

Placement of oesophagostomy tube	Oesophagostomy tube placement is discussed in the Hospitalisation chapter. It requires analgesia and/or local or general anaesthesia.
Debridement of ocular lesions associated with hypovitaminosis A	This is illustrated in Figs 15.154–15.156.
Management of stomatitis	• Where severe cases of virus-associated or other stomatitis are presented, debridement of necrotic diphtheroid lesions under general anaesthesia is usually beneficial. • The patient should first be stabilised and any dehydration and sepsis addressed. Anaesthesia and analgesics should be provided. • Diphtheroid lesions are gently stripped away using cotton buds and forceps. • Samples suited to viral PCR, virus isolation, electron microscopy, cytology, immunocytology, histopathology and histochemistry can be harvested during the procedure. • Most cases will benefit from simultaneous oesophagostomy tube placement.
Scute debridement	• Animals presenting with shell trauma and chronic shell infections will benefit from the removal of infected and necrotic material. • Many scutes will simply lift off following flat insertion of a scalpel blade, which can then flick the scute off. • Remove all abnormal scutes and all abnormal material at the margins of lesions. Often this means that debridement at the lesion periphery must be fairly aggressive. • At the edges of lesions and where lesions are active, it is important to provide anaesthesia and/or analgesia before removing significantly-attached scutes. • Samples to determine the nature of any mycotic or bacterial agents present should be harvested and analysed. • Following collection of diagnostic material, exposed tissues are generally cleaned with a disinfecting solution such as dilute povidone-iodine.
Placement of cardiac port	This is described earlier in the Cardiocentesis section of Venepuncture. General anaesthesia is required and the procedure is generally restricted to experimental work at research establishments, where regular cardiac access is required for administration of medication or serial blood sampling. A cardiac access port would give the capability of administering cytotoxic drugs to chelonians that have a diagnosis of neoplastic disease with minimal operator risk, although such work would be considered experimental.
Placement of vascular access port	As with a cardiac port, a subcutaneous or carapace-secured vascular access port allows serial blood samples to be taken or regular intravenous medications to be given. It is beyond the scope of general practice but has potential applications in research establishments, where experimental work is undertaken or neoplasia is treated with cytotoxic agents.
Skin biopsy	Whilst simple cytology of skin lesions can be undertaken in the conscious animal, occasional skin lesions will be much better addressed following full-thickness biopsy and appropriate analysis. It is prudent to place some material in formol saline for histopathological examination, to send some for simple microbial culture and sensitivity examination and to retain some should viral or mycobacterial analysis be required following histopathology results.
Shell biopsy	Shell biopsy can be a useful tool in the diagnosis of mycotic (e.g. terrestrial species) or bacterial (e.g. semi-aquatic species) infections. Often scute loss and infection relate to inappropriate care and hygiene and an animal with scute abnormalities and sloughing will also require a more general work-up and health assessment. Neoplastic disease of the plastron was diagnosed by Cooper *et al.* (1983) following shell biopsy. The tumour occurred at the site of a previous shell infection associated with *Proteus morganii*, however it is unknown if the initial infection was itself secondary to neoplasia. Lawton (1996) describes partial plastronectomy in the management of deep shell infections in *Testudo* spp.
Shell conformation alterations.	Excessive growth of the shell is common in juvenile animals experiencing accelerated growth as result of unsuitable husbandry and nutrition. Techniques for resecting the shell are illustrated in Figs 15.157–15.162.
Limb amputation and excision arthroplasty	See Figs 15.163–15.166.
Wheel attachment	Wheels have been applied to chelonians by a number of authors. They have uses in the post operative management of limb amputations, in cases of hind-limb paresis and where the animal has good forelimb function but very poor hind-limb function (Figs 15.167–15.171). As wheels can become entangled in grass, a billiard or snooker ball cut in half can be glued to the carapace as an alternative.

Fig. 15.156 The eye is then irrigated and an antibiotic preparation applied. Underlying problems with inappropriate nutrition should be corrected.

Fig. 15.157 Overgrowth of the plastron in a juvenile *Testudo marginata*. This animal was unable to void faeces without contaminating the skin adherent to the dorsal plastron, which therefore suffered a chronic dermatitis.

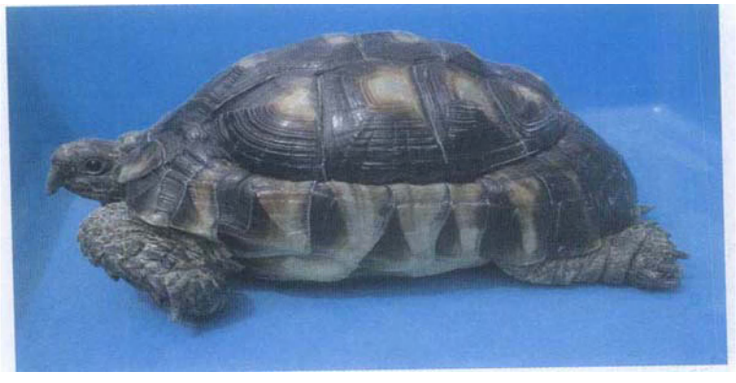


Fig. 15.158 Same case as Fig. 157: The animal displayed typical signs of accelerated growth. In this case the growth rate of the plastron and ventral tissues were vastly in excess of that of the carapace and dorsal tissues. This may be partly explained by the sole use of an inappropriate ventral heat source.

Fig. 15.159 Using wooden tongue depressors to shield sensitive tissues, the shell was burred back under general anaesthesia.

Pre-operative antibiotics and analgesics were administered.

Fig. 15.160 A rotating diamond burr was used to cut back the shell. Perhaps surprisingly, bleeding was limited and no specific steps were necessary to control haemorrhage.

Analgesia and antibiotic cover were provided throughout the healing period.

Fig. 15.161 A stone polishing head was used to smooth the shell.

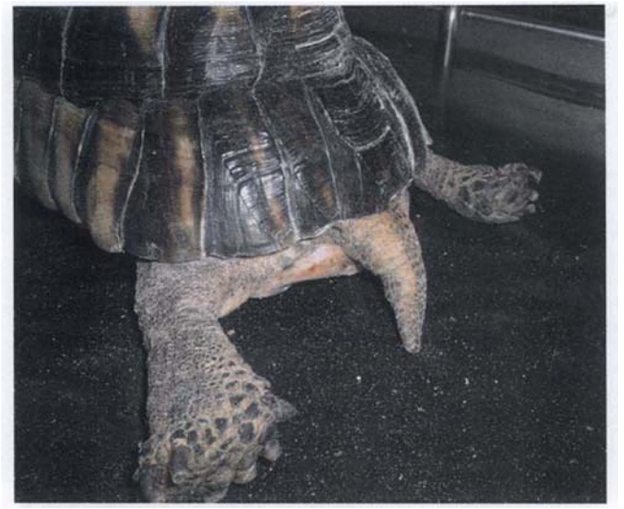


Fig. 15.162 Following surgery, relatively normal anatomy was regained.

Fig. 15.163 Stump necrosis in a referred spur-thighed *tortoise* (*Testudo graeca*) one year after limb amputation.

Fig. 15.164 Surgical exploration revealed extensive osteomyelitis of the residual femoral bone, which was removed.

Fig. 15.165 The femoral head and associated caseous material following removal.

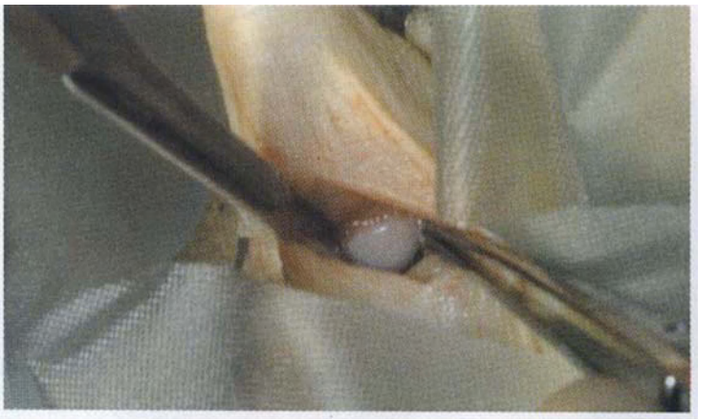


Fig. 15.166 Excision arthroplasty in a juvenile *Geochelone sulcata*. (Courtesy of Aidan Raftery)

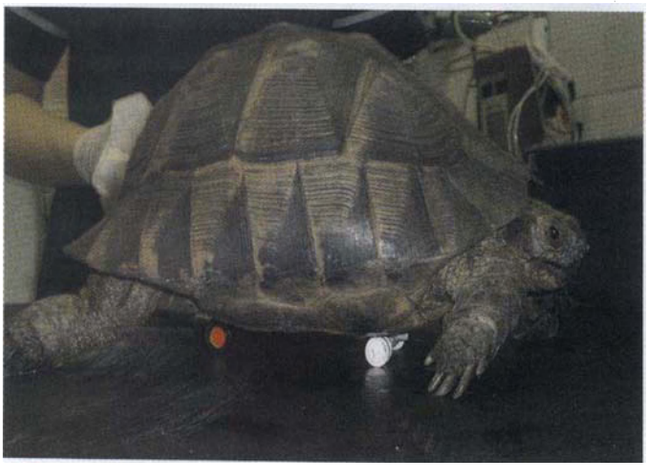


Fig. 15.167 It may take some time to find the right size wheels to allow a chronically immobile chelonian to move freely once again.

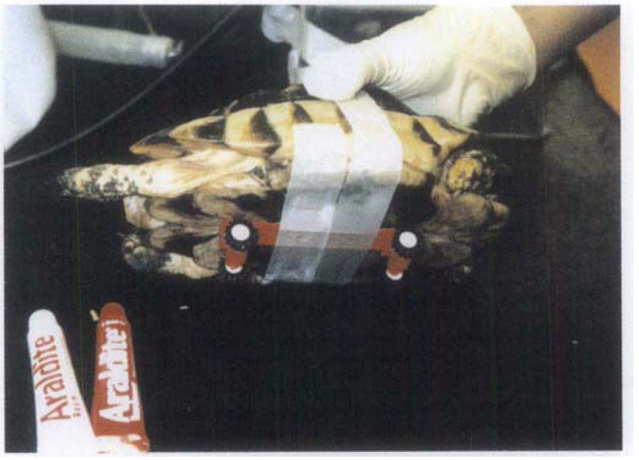


Fig. 15.168 Here, wheels have been applied in a fashion similar to a skateboard. A Lego® base plate is attached to the plastron using an epoxy such as that illustrated. This can be removed and replaced as the animal grows. The wheels are pressed into the plate but not glued, so that they can be detached overnight, or will come off if snagged. This *Testudo hermanni* has metabolic bone disease and pelvic distortion.

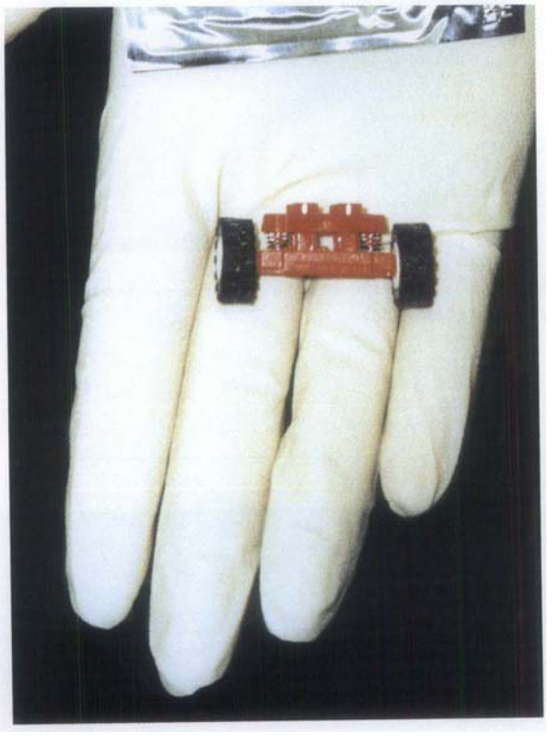


Fig. 15.169 Close up of Lego® wheels regularly used by the author. These have suspension springs that maintain the ground contact of the wheels as the animal rocks from side to side during normal walking. The wheels shown are also a size appropriate to chelonians encountered commonly in UK practice. Wheels can be regularly replaced as they wear out.

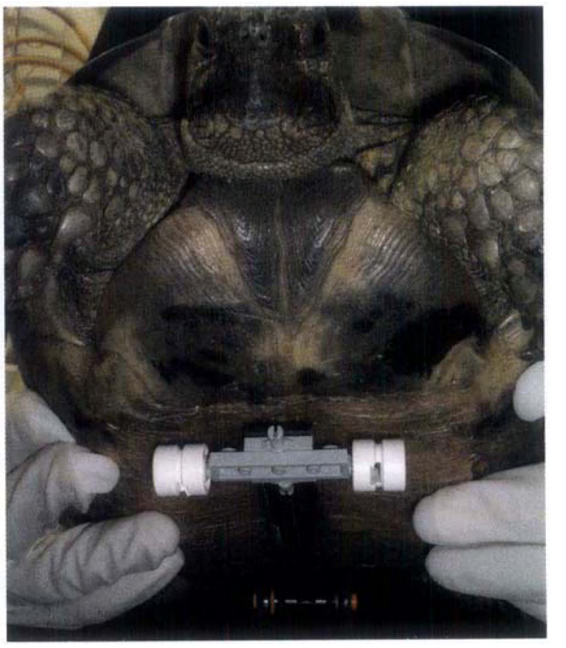


Fig. 15.170 In order to allow normal range of movement it is necessary to apply wheels to maintain the centre of gravity in a relatively natural position. In most cases any wheels positioned at the front of the animal will need to be smaller than those placed towards the rear.

Fig. 15.171 Location of the wheels is crucial and animals should be hospitalised for a short while in order to observe them once the wheels are applied. In cases of chronic hind limb paresis, the animal may easily raise the front of its body, but may need wheels as a skid to allow free movement of its hind end. Animals should be provided with flat areas allowing suitable grip (e.g. rubber pond liners laid on concrete).

THERAPEUTICS

ROGER WILKINSON

INTRODUCTION

The agents discussed below, with the exception of enrofloxacin (Baytril®, Bayer), are not licensed for use in chelonians in the United Kingdom. The clinician should be aware that very limited information is available at the time of writing and that variables such as species, route of administration, hydration status and metabolic changes in disease might unpredictably affect the safety and efficacy of therapeutic agents used in chelonians. Whilst every care has been taken to ensure that the information presented here is accurate, no responsibility can be taken by the authors should any legal matter arise from the use of this information.

We advise that clients be asked to sign a consent form for the use of an unlicensed product before any animal is treated.

TEMPERATURE AND THERMOTHERAPY

It is essential that chelonian patients be maintained within their appropriate temperature range (ATR). Many believe that the upper end of this range is best. Large patients may require a significant period, perhaps extending to days, to achieve an optimum temperature. Immune system function is strongly temperature dependent and sub-optimal temperature is an important factor in the causation of many disorders. Indeed, some conditions may respond to the correction of hypothermia alone without further treatment, and sick reptiles themselves may exhibit *behavioural fever*—seeking out warmer conditions than normal. *Thermotherapy* (Ross 1984) takes this concept one step further, aiming to maintain the patient at the top end of its ATR.

This technique is discussed critically by Jacobson (1999a). It should be borne in mind that although reptiles may at times seek temperatures at the top end of the ATR, prolonged exposure to such temperatures can cause a decline in appetite and body weight. Highfield (1996), for example, states that Moroccan tortoises (*Testudo graeca graeca*) cease activity at temperatures above 28°C. In the wild, Horsfield's tortoises *Testudo* (*Agrionemys*) *horsfieldi* also aestivate in the hottest summer months. Allowance should also be made for increased water loss from patients maintained at higher temperatures. Smaller individuals lose water more rapidly by evaporation than do larger patients (Bentley 1976).

Temperature is also an important factor in drug therapy. Although higher temperatures do not per se necessarily enhance antibacterial activity, they may facilitate distribution of drug within the body, thus achieving higher concentrations at the site of infection. Higher temperatures generally necessitate higher and more frequent doses of drug to maintain tissue levels. However, potential for toxicity may actually be increased in some cases and this should be borne in mind. It is important that pharmacokinetic studies should specify the temperature at which the trial was conducted.

Pokras *et al.* (1992) present a formula for use in reptiles which is designed to allow a clinician to judge whether or not a patient has reached its preferred body temperature:

$$\text{Heart rate at preferred body temperature (PBT)} = 34 \times (\text{body weight in kg})^{-0.25}$$

However, no data is presented to substantiate this relationship. It is not clear what criteria were used to define preferred body temperature in formulating this equation or whether variables such as sickness, hydration status, electrolyte balance or cardiac disease might affect its reliability in practice

CALCULATING DRUG DOSAGES AND INTERVALS

Evidence presented by Bennett & Dawson (1976) and Lawrence (1984b) suggests that the weight of the shell should not be subtracted when calculating drug doses for chelonians. This is in contrast to earlier assumptions that the shell is metabolically inert (Hughes *et al.* 1971).

Pharmacokinetic data is scarce. For example, there is just one published pharmacokinetic study relating to sea turtles (Stamper 1997). It is therefore often necessary to extrapolate from the most comparable published study. In such a situation the considerations outlined in Table 16.1 should be taken into consideration.

Encouragingly, Lawrence (1983) demonstrated that minimum inhibitory concentrations (MICs) of antibiotics for bacteria isolated from reptiles were comparable to those for equivalent human isolates. It appears that the optimum tissue level of antibiotic, at least, can be extrapolated from mammals to reptiles.

ROUTES OF DRUG ADMINISTRATION

Drugs may be administered to chelonians by a number of different routes. These are summarised below.

Oral

The practicalities of oral drug administration are described in nursing techniques. It is important to bear in mind that the volume of drug to be administered is limited by stomach capacity. In the past, oral dosing has been frowned upon for a variety of reasons, although little pharmacokinetic work had been published until recently. The only firm guidance available was the work of Bush *et al.* (1976) who showed that chloramphenicol was absorbed slowly from the gut.

- Gastrointestinal transit times vary widely and were thought to make absorption unreliable. The nature of the diet is important in this respect. In general, passage is more unpredictable in herbivores.

Table 16.1 Factors to consider when calculating drug dosages.

Mammals vs. reptiles	Drug half-lives are generally longer in reptiles than mammals (Lawrence 1983).
Chelonians vs. other reptiles	An analysis of the very sparse published pharmacokinetic data supports the contention that doses for chelonians should be of the same order of magnitude as for lizards and snakes. That is to say that the differences between members of separate orders of reptiles are broadly comparable with inter-species differences between chelonians.
Water economy	At present we do not have enough studies available to say whether doses and dose frequencies should generally be higher for freshwater chelonians than for marine or terrestrial species when using renally-eliminated drugs, but this possibility should be borne in mind. Hodge (1978) found that, in a largely-aquatic freshwater snake, elimination of gentamicin was more rapid than in terrestrial species.
Body size	The kinetics of uptake, distribution and elimination correlate better with metabolic rate than with body weight. Smaller reptiles often require higher mg/kg doses at more frequent intervals than larger ones (Mader *et al.* 1985). Mammals, on the whole, conform to a single equation relating metabolic rate (and hence drug-dosage requirements) to body mass. Metabolic rate is often represented as minimum energy cost (MEC), a representation of basal metabolic rate, or specific minimum energy cost (SMEC), a representation of basal metabolic rate per unit of body weight. For reptiles, however, this relationship may not be so easy to formulate (Hainsworth 1981; Schumacher & Jacobson 1995; Jacobson 1999a). No validated equation has yet been suggested for chelonians. The concern is that it may prove difficult to produce widely-applicable formulae given the existence of unpredictable variables such as species idiosyncrasies, age, hydration status, temperature and metabolic differences between sick and healthy animals. At present, it must be concluded that metabolic scaling for chelonian medicine remains an interesting possibility rather than a day-to-day reality.
Temperature	Body temperature not only affects dose and dose interval but may also alter drug distribution (Mader *et al.* 1985). Higher temperatures generally necessitate more frequent administration of drugs. The same study revealed that, for amikacin, higher temperatures may lead to *reduced* dose requirements and thus reduced risk of toxicity. The issue of temperature is discussed further below.
Hydration status	Renally excreted drugs appear to be eliminated faster when hydration status is maintained. Bush *et al.* (1980) found that, when 2.2 ml/kg of lactated Ringer's solution was administered subcutaneously at the same time as gentamicin 5 mg/kg/day, plasma levels after five days were 18 mg/l as opposed to 47 mg/l when gentamicin alone was given. The problem is compounded, as in this example, when the drug is also potentially nephrotoxic.
Frequency of urination	Renally-excreted drugs are potentially available for reabsorption from the bladder when urine is not voided and may be more slowly eliminated as a result (Lawrence *et al.* 1986). This is partially a function of hydration status but other triggers, such as handling or bathing, may prompt urination regardless of water balance. This is at present a theoretical concept.
Nature of pharmacokinetic study	Wimsatt *et al.* (1999) highlighted the differences between data extrapolated from single-dose pharmacokinetic studies and that from multi-dose studies. Many of the published accounts relate to single-dose trials. Information from multi-dose studies must be preferred.
Species idiosyncrasy	Unpredictable species differences may arise. For example, Aucoin (1991) found that, in box turtles (*Terrapene carolina*), a 5 mg/kg intramuscular dose of enrofloxacin produced prolonged blood levels, while Spörle *et al.* (1991) demonstrated that 10 mg/kg administered to *Testudo hermanni* led to therapeutic plasma concentrations enduring for only 24 hours.

- Drugs must pass through the liver after absorption before entering the systemic circulation. Exposure to hepatic pre-systemic elimination is thus potentially increased for hepatically-excreted or metabolised drugs.
- It may be difficult to medicate large, strong species. Giant tortoises require anaesthesia and oesophagostomy tube placement (Norton *et al.* 1989). Sea turtles have long papillae lining the oesophagus, which trap food whilst water is expelled, and these complicate the passing of a stomach tube.

On the other hand, more recent pharmacokinetic studies have renewed interest in oral medication. Vancutsem *et al.* (1990) found that enrofloxacin may be almost completely absorbed whilst Wimsatt *et al.* (1999) described the encouraging pharmacokinetics of orally administered clarithromycin in desert tortoises *Gopherus agassizii*. Pharmacokinetic data is available for orally administered ketoconazole (Page *et al.* 1991).

There are also positive advantages of oral dosing:

- This method may be applicable to large groups. Jacobson *et al.* (1983) successfully medicated large numbers of red-footed and leopard tortoises (*Geochelone carbonaria* and *G. pardalis*) with dimetridazole in food to alleviate amoebiasis. In-food medication may be the only practical way of treating groups of sea or freshwater turtles.
- Gastrointestinal infections and infestations are most logically treated orally.
- Oral fluid therapy offers reduced risk of fluid overload.
- In easily-handled animals, or after the implantation of an indwelling oesophagostomy tube, oral medication offers positive advantages over injection. Injections are painful, more difficult for owners to administer and involve more risk of adverse effect.

The nature of the drug to be administered should be considered before choosing this route. Tetracyclines, for example, would generally be a poor choice for oral administration since they

may bind to gut contents—particularly if a calcium-containing supplement is being given.

Per-cloaca/colon

In many vertebrates, drugs are well absorbed from the rectum. Based on a limited number of cases, Innis (1997a) recommended per-cloacal administration of fenbendazole in the treatment of oxyurid infestation in chelonians. These nematodes live in the large intestine and do not attach to the gut wall. They are thus relatively protected from orally or parenterally administered anthelminthics.

Cloacal dosing is relatively easy and offers a particularly attractive option in uncooperative species such as leopard tortoises (*Geochelone pardalis*). The volume that can be given in this way is limited, and there is always the possibility of the drug being voided before therapeutic effect is achieved.

Little is known of drug pharmacokinetics for colonically administered drugs in chelonians. However, it seems fair to assume that fluids will be well absorbed.

Antibiotic-impregnated polymethyl-methacrylate (PMMA) beads

Implantation of antibiotic-impregnated beads is most applicable to the treatment of osteomyelitis or septic arthritis (Divers & Lawton 1999). The technique offers the dual advantages of good drug delivery to the infected site and reduced chance of systemic toxicity. Aminoglycosides and clindamycin are the drugs most often used in this way. Divers & Lawton suggest 2 g neomycin plus 2 g clindamycin per 20 g of PMMA and reported finding no nephrotoxicity with this recipe. Beads may be prepared in a sterile fashion or may be gas-sterilised after preparation. General principles of antibiotic use should be followed (culture and sensitivity in advance, surgical debridement where possible etc.).

Intrapneumonic (*Divers 1998*)

Drugs may be injected into the lungs via the inguinal fossa with a long needle directed craniodorsally. Transtracheal injection is also possible in larger animals. In cases of focal disease, an intrapneumonic 18 G–22 G catheter may be surgically positioned within the lesion via a hole drilled in the carapace and secured with tissue glue. Potentially toxic medications such as amphotericin B or gentamicin may thus achieve high tissue concentrations, whilst the risk of toxicity may be reduced, since these drugs are thought to be poorly absorbed across respiratory epithelium. Divers (1998b) also suggested dose rates for intrapneumonic therapy in animals maintained at 25°C–30°C based on his experience:

Table 16.2 Suggested intrapneumonic doses of antibiotics.

Drug	Intrapneumonic dose rate
Amphotericin B	1–2 mg/kg every 24 hours
Gentamicin	5 mg/kg every 48 hours
Amikacin	5 mg/kg every 48 hours
Enrofloxaim	5 mg/kg every 24 hours
Doxycycline	10 mg/kg every 24 hours

Intravenous, intraosseous, intracoelomic injection

Injection sites and techniques are described in detail in nursing techniques.

In summary:

- Intravenous injections are technically challenging and are little used except perhaps in sea turtles, which have more readily-discernible venous access points.
- Intraosseous drug administration can be regarded almost as equivalent to intravenous therapy and is a very useful technique—particularly in critically-ill individuals.
- Relatively large volumes of fluids (see below) can be given with minimum technical difficulty (and thus minimum stress—see discussion of lactic acidosis below) into the coelomic cavity of most chelonians. Antibiotics may also be given effectively by this route for the treatment of coelomitis (for example, post-coeliotomy). A theoretical risk of impairing respiratory function exists when larger volumes are introduced into the coelomic cavity (see fluid therapy, below); however, up to 5% of body weight is probably safe.

Intramuscular injection

There is a limit to the volume of fluid that can be given intramuscularly. Even using the pectoral muscles, we do not exceed a few ml/kg body weight. More consideration must be given to the chemical nature of the solution for injection. Bases high in cations such as potassium may exacerbate electrolyte imbalances or trigger precipitation of urates in hyperuricaemic animals. Some preparations also seem to be painful.

Subcutaneous injection

The subcutaneous route is probably sub-optimal for fluid therapy. Fluids may be slowly absorbed, particularly by dehydrated animals with compromised peripheral circulation. If the situation is not urgent, then oral or colonic rehydration may be preferable. Possible irritation by some drugs is probably even more important here. Some enrofloxacin preparations, for example, have caused permanent skin changes in reptiles. Glucose solutions exceeding 2.5% should not be given subcutaneously.

RENAL PORTAL SYSTEM

The renal portal system is discussed in detail by Holz (1999) and in the chapter on Anatomy and Physiology. All reptiles possess a renal portal system, which carries a proportion of blood (the exact amount is controlled by valves) from the hind limbs and tail through the kidneys on the way back to the heart (Holz *et al.* 1997). In this way, the metabolic needs of the kidneys are met when their arterial flow is restricted in the interests of water conservation. Renal-portal blood mixes with arterial blood perfusing the proximal and distal convoluted tubules but not the glomeruli. This phenomenon is potentially of relevance because drugs that are eliminated at the tubular level might suffer accelerated excretion when administered in the caudal part of the body.

Antibiotics, such as most aminoglycosides, cephalosporins and penicillins, which are largely excreted by the kidneys, include

some of the most important weapons in our armoury. However, the clinical relevance of this problem may be minimal: Beck *et al.* (1995) and Holz *et al.* (1994) found that no significant difference in drug metabolism was seen when gentamicin (which, like all aminoglycosides, is excreted by glomerular filtration) was injected into the hind limb rather than the forelimb. For carbenicillin (a significant proportion of which is actively secreted by the tubules) blood levels were slightly lower for the first eight hours in the hind-limb-injected group but the authors noted that blood levels remained well in excess of the MIC for relevant pathogens, despite the use of a dose half that recommended by Lawrence *et al.* (1986). It was concluded by Holz (1999) that the proportion of blood directed through the renal-portal system is unlikely to be of clinical significance even in dehydrated animals.

There is no firm evidence, at present, to support the contention that drugs should not be injected into the hind limbs or tail of chelonians. However, research is in its infancy. All other things being equal, cranial sites should be used.

ANTIBACTERIALS

The following general considerations should be borne in mind:

- Granulomas, which are poorly penetrated by antibiotics, are common in reptiles (Montali 1988). Surgery must be considered as a primary measure in these cases. Antibiotic therapy is used thereafter.
- Because antibiotic resistance is a strong possibility with members of the Enterobacteriaceae, which comprise the most common pathogens of chelonians, it is especially important to collect pre-treatment samples for aerobic and anaerobic culture and sensitivity. Where mycobacterial infection is a possibility, retain a (non-formalinised) sample for subsequent specific mycobacterial culture. Consider the use of blood cultures in very sick, potentially septicaemic, patients. Sensitivity tests for ceftazidime, amikacin, gentamicin and enrofloxacin in particular should be requested.
- In some circumstances, for initial treatment or where no agent can be cultured, it may be necessary to select an antibiotic empirically. Antibiotic sensitivity patterns for isolates from reptiles have been reviewed (Burke *et al.* 1978; Needham 1981). The bacteria responsible are often Gram-negative opportunists although a variety of organisms have been implicated. The cutaneous microflora of chelonians is rich in members of the family Enterobacteriaceae, such as *Pseudomonas, Proteus, Aeromonas* (especially in aquatic chelonians), *Providencia, Morganella, Salmonella* and *Klebsiella*.
- Stock & Wiedemann (1998) reviewed the antibiotic susceptibility of *Morganella*, which is typical of this group in many respects. The natural population of *M. morganii* is primarily (naturally) resistant to certain penicillins such as benzylpenicillin, oxacillin, and amoxicillin, first and second generation cephalosporins (excluding cefoxitin), cefpodoxime, all antibiotics of the ML group (macrolides and lincosamides), sulfamethoxazole, glycopeptides, fosfomycin, and fusidic acid. They are naturally sensitive to aminoglycosides, piperacillin, mezlocillin, ticarcillin, third and fourth generation cephalosporins, carbapenems, aztreonam, quinolones, trimethoprim, cotrimoxazole, and chloramphenicol.
- Enrofloxacin (a quinolone) and ceftazidime (a third-generation cephalosporin) are probably the best choices for our patients in this situation since they are relatively safe and we know something of their pharmacokinetics. Enrofloxacin has the notable disadvantage that it is relatively ineffective against anaerobes.
- Culture alone will not prove that any agent identified is involved in the pathogenesis of the disease process. Chelonian bacterial pathogens are usually opportunists and can readily be isolated from healthy animals. For this reason, cytological or histological evidence of pathogenicity is also very important.
- Bactericidal agents are theoretically preferable because immune-system function may be sub-optimal in many patients. Suitable antibiotics include β-lactams, quinolones, aminoglycosides, potentiated sulphonamides and metronidazole.
- The possibility of counter-productive endotoxin release with aggressive anti-Gram-negative antibiotic therapy has been suggested (Holt 1981). However, this remains a theoretical concept. In human medicine the priority is very much the elimination of pathogens. Beta-lactams are generally associated with greatest endotoxin release, fluoroquinolones with less and aminoglycosides with least.
- Anaerobes are increasingly recognised as important reptile pathogens (Stewart 1990). Metronidazole, chloramphenicol and many (but not all) β-lactam antibiotics are potentially effective in treating such infections.
- *Pasteurella* is more often isolated from the nasal cavity of desert tortoises (*Gopherus agassizii*) with apparent mycoplasmosis than from healthy animals (Jacobson *et al.* 1991b). Antibiotics effective against *Pasteurella* may be useful in the treatment of upper respiratory tract disease.
- Anti-*Mycoplasma* activity is an advantage in certain situations. Mycoplasmosis is much less frequently diagnosed in Europe than in the United States, but is believed to play a role in the pathogenesis of upper respiratory tract disease in some species. Antibiotics effective against *Mycoplasma* include clarithromycin, tylosin and enrofloxacin.
- *Mycobacteria* are regularly isolated from cutaneous, subcutaneous and visceral (pyo) granulomatous lesions in chelonians. They present a special challenge. Surgical resection should be attempted where possible. Antibiotics with anti-mycobacterial efficacy include enrofloxacin, clarithromycin and doxycycline, although *in vitro* sensitivity studies should be conducted where possible. Combination therapy in long courses is the rule in man. We have achieved long-term cure in one subcutaneous mycobacterial abscess with the use of surgery followed by two months of parenteral enrofloxacin.
- Some drugs are potentially expensive in large species.
- Chelonia have no eye spectacle and do not present the special problems of sub-spectacular infection seen in snakes and some lizards—on the contrary, they often have spectacular infections!

Beta-lactam antibiotics

To summarise the major characteristics of β-lactam antibiotics:

- They are bactericidal.
- Many have good anti-anaerobe activity. Cloxacillin and dicloxacillin are examples of exceptions.

- They are largely excreted by the kidneys (with notable exceptions e.g. cefoperazone).
- They are generally active in the presence of pus and organic debris (Bryant and Hammond 1974).
- Semi-synthetic penicillins, such as carbenicillin, piperacillin, mezlocillin and ticarcillin have increased activity against Gram-negative bacteria. In particular, the latter three are often described as *anti-pseudomonal* penicillins.
- They may also be used in combination with a β-lactamase inhibitor, such as clavulanate.
- They are relatively non-toxic.
- They are more vulnerable to the development of resistance than aminoglycosides.
- It has been suggested that pain on injection may occur with this entire group (Klingenberg 1996a).

Table 16.3 summarises the β-lactam antibiotics.

Aminoglycosides

The aminoglycosides form a group including amikacin, gentamicin, netilmicin, tobramycin, kanamycin, neomycin and streptomycin.

They are:

- bactericidal;
- largely renally-excreted;
- broad spectrum, with the notable exception of anaerobes which are uniformly non-susceptible to aminoglycosides;
- reduced in activity in the presence of pus (Bryant and Hammond 1974);
- potentially nephrotoxic, ototoxic and even cardiotoxic;
- anti-pseudomonal: amikacin and tobramycin > gentamicin > netilmicin;
- nephrotoxic: gentamicin > amikacin and tobramycin > netilmicin.

Table 16.4 summarises the members of this group.

Chloramphenicol

Chloramphenicol is:

- bacteriostatic;
- absorbed slowly after oral administration and failed to achieve therapeutic serum concentrations, according to Bush *et al.* (1976);
- metabolised predominantly in the liver;
- broad spectrum, including anaerobes, but has poor anti-pseudomonal effect, although *Proteus* may be sensitive;
- widely-distributed within the body.

A very depressed PCV was seen in one chloramphenicol-treated snake (Clark *et al.* 1985). This may represent the same kind of haematological abnormality occasionally associated with chloramphenicol use in mammals.

It should be borne in mind that fluoroquinolones, for example, are also broad spectrum (excluding anaerobes) and widely distributed but have less toxic potential and good anti-pseudomonal activity. In addition, there is more pharmacokinetic data available for chelonians. However, despite its disadvantages, chloramphenicol has been used successfully in reptiles. Kaplan (1957) considered chloramphenicol to be the treatment of choice for septicaemic cutaneous ulcerative disease (SCUD) in freshwater turtles.

Tetracyclines

Tetracyclines have limited application in the treatment of the Gram-negative infections that predominate in chelonians. However their mode of activity gives them potential applications in the treatment of less common infections such as mycoplasmosis, chlamydiosis (Homer *et al.* 1994), mycobacterial infections and possibly haemoparasites, such as *Haemoproteus*. Doxycycline is used as an anti-malarial in man.

The main characteristics of tetracyclines are:

- bacteriostatic;
- potentially unpredictable absorption after oral administration;
- may bind to calcium salts and should not be orally administered at the same time as mineral supplements;
- hepatic metabolism and excretion;
- widely distributed in the body;
- anti-mycobacterial effect (doxycycline);
- anti-chlamydial effect;
- anti-mycoplasma effect.

Table 16.5 summarises the main tetracyclines.

Fluoroquinolones

Fluoroquinolones are:

- varied: ciprofloxacin, norfloxacin and enrofloxacin are available;
- safe;
- bactericidal (the bactericidal activity of enrofloxacin is concentration dependent);
- broad-spectrum: including Enterobacteriaceae such as *Pseudomonas*;
- anti-mycoplasmal;
- anti-mycobacterial;
- anti-chlamydial;
- well absorbed after oral or parenteral administration (Vancutsem *et al.* 1990);
- widely-distributed within the body—including the eye and central nervous system in man.

Although obligate anaerobes are resistant, this may be an advantage if no anaerobes are involved in the disease process, since gut flora will be preserved.

Fluoroquinolones may cause pain, inflammation and local tissue necrosis (subcutaneous injection) on injection (Table 16.6).

Macrolides

Macrolides may be bacteriostatic (tylosin, erythromycin) or bactericidal (clarithromycin, azithromycin) although this depends upon drug concentration and microbial sensitivity. They are relatively safe (Table 16.7).

Lincosamides

Clindamycin

Nutter *et al.* (2000) used this drug with apparent success at 5 mg/kg IM every 24 hours for 14 days in the treatment of *Clostridium difficile* infection of the reproductive tract and coelomic cavity in a loggerhead sea turtle (*Caretta caretta*).

Drug	Characteristics
Carbenicillin	• Lawrence *et al.* (1986) found that an intramuscular dose of 400 mg/kg of carbenicillin lead to a biphasic increase in serum levels. It was hypothesised that the drug, which is renally excreted, was being reabsorbed from the bladder. The dose was found to be safe and effective when repeated every 48 hours. • Carbenicillin was effective *in vitro* against 23/32 of bacterial isolates from reptiles (Lawrence 1984a). At Holly House surgery, United Kingdom, 20/22 bacterial isolates from chelonian patients were carbenicillin sensitive *in vitro*. • A rash has been associated with the use of carbenicillin in desert tortoises (Rosskopf 1999). • Carbenicillin is vulnerable to the rapid development of resistance in human use. We have seen chelonian cases where bacteria have been susceptible at the outset and resistant two months later. For this reason it is best used in combination with other antibiotics, such as an aminoglycoside or fluoroquinolone. • The relationship between gentamicin and carbenicillin is a complicated one. Lawrence (1983) reviews the literature on this subject, citing material which suggests that combination therapy is clinically effective although the two antibiotics may chemically inactivate each other (Riff & Jackson1972) and should be injected into separate sites. Jacobson (1999a) describes the use of a protocol where the dose of gentamicin or amikacin is given every third day, followed on each occasion by carbenicillin or piperacillin 48 hours later.
Ampicillin	• 12/22 bacterial isolates from chelonian patients at Holly House surgery, United Kingdom, were ampicillin sensitive *in vitro*. • Lawrence (1984a) found that only 4/32 bacterial isolates from reptiles were ampicillin sensitive. • In *Testudo hermanni*, 50 mg/kg IM bid was found to be a sufficient dose to achieve blood levels effective against *Staphylococci* although not *Pseudomonas* or most *Klebsiella* and *Salmonella* strains (Spörle *et al.* 1991). • Lawrence (1983) found that most Enterobacteriaceae were ampicillin-resistant, except for *Salmonella arizona* and some *Proteus mirabilis*. • Ampicillin may be a good choice for the treatment of dermatophilosis (Hermoso de Mendoza *et al.* 1994).
Piperacillin	• High activity against many Gram-negative pathogens, including *Pseudomonas*. • Also available with the β-lactamase inhibitor tazobactam (Perry & Markham 1999). • No data is available for chelonians. However, in pythons, doses of 100 mg/kg IM every 48 hours led to blood levels greatly exceeding the MIC of important pathogens (Hilf *et al.* 1991).
Ceftazidime	• Ceftazidime is a third-generation cephalosporin. • 16/22 bacterial isolates from chelonian patients at Holly House surgery, United Kingdom, were ceftazidime sensitive *in vitro*. • Ceftazidime is often used in chelonians although most pharmacokinetic data stems from work in snakes. • In juvenile loggerhead sea turtles (*Caretta caretta*), Stamper *et al.* (1997) showed that plasma levels remained above the MIC for *Pseudomonas* for 60 hours after a single intramuscular or intravenous dose of 20 mg/kg. • 20 mg/kg IM every 72 hours is the recommended dose at 30°C in snakes (Lawrence 1984). Peak plasma levels occurred eight hours after an intramuscular dose. The biological half-life was approximately 24 hours with therapeutic levels being maintained for up to 96 hours. Several snakes treated with 20 mg/kg every 72 hours 'showed an excellent clinical response'. • Ceftazidime was found to be 32 times more effective against *Pseudomonas in vitro* than carbenicillin (Clark & Zemcov 1981) although some strains of Enterobacteriaceae are still resistant (Lawrence 1984a). • Jacobson (1999a) states that he has 'never observed negative side-effects of ceftazidime in reptiles'. • Ceftazidime is widely distributed in the body and, in man, achieves therapeutic concentrations in bone, bile, skin and kidney.
Cefoperazone	• Cefoperazone is a third-generation cephalosporin. • It is more lipophilic than ceftazidime and is thus more widely distributed within the body. • Cefoperazone is eliminated principally by hepatic metabolism rather than renally, as is the case for ceftazidime. This is potentially an important consideration given the incidence of renal compromise in sick chelonians. • It is possibly less susceptible to ß-lactamase action than ceftazidime. • The recommended dose in Tegu lizards (*Tupinambis teguixin*) is 125 mg/kg every 24 hours, and, in a colubrid snake, 100 mg/kg every 96 hours (Klingenberg 1996a).
Ticarcillin	To the author's knowledge, nothing has been published on the use of this carboxypenicillin in reptiles. When conjugated with clavulanic acid (Timentin®, Beecham) to resist β-lactamase, it is widely used in human medicine to treat *Pseudomonas* infections and undoubtedly has potential for chelonian patients.

Table 16.4 Aminoglycosides.

Drug	Characteristics
Amikacin	• There appear to be no references to nephrotoxicity for amikacin. • Jacobson (1999a) says that he has seen isolates resistant to amikacin (*in vitro*) and sensitive to gentamicin. It is not true to say that amikacin is universally preferable to gentamicin. • Caligiuri *et al.* (1990) found that, in the gopher tortoise (*Gopherus polyphemus*), the clearance rate was twice as fast at 30°C as at 20°C. They recommended 5 mg/kg IM every 48 hours at 30°C. • Mader *et al.* (1985) found that at 37°C the dose required to achieve effective serum concentrations was less than at 24°C—thus reducing the potential for nephrotoxicity.
Gentamicin	• 16/22 bacterial isolates from chelonian patients at Holly House surgery, United Kingdom, were gentamicin sensitive *in vitro*. • Its potential for nephrotoxicity is well-established (Montali *et al.* 1979). Gentamicin-induced visceral gout has been seen in snakes (Jacobson 1976). Hodge (1978) demonstrated a linear relationship between dose and blood uric-acid levels in snakes. • Under some circumstances, it may reduce the risk of nephrotoxicity to give fluids before or concurrently with gentamicin. Bush *et al.* (1980) found that when 2.2 ml/kg lactated Ringer's solution was given at the same time as gentamicin (5 mg/kg/day) plasma levels after five days were 18 mg/l as opposed to 47 mg/l when gentamicin alone was given. However, if the animal is not dehydrated then there must be a risk of accelerated elimination and reduced efficacy. If dose rates used are derived from studies where fluids were not administered, then the patient should be critically assessed with respect to hydration status rather than routinely given fluids. • Bush *et al.* (1977) reported the half-life for subcutaneously injected gentamicin in the freshwater turtle (*Chrysemys picta*) to be 32 +/– 15 hours at 26°C. The suggested dose was 10 mg/kg IM every 48 hours. Therapeutic serum levels were achieved within eight hours, however, it was calculated that toxic levels would have accumulated by day 15. • In the related *C. scripta elegans*, Raphael *et al.* (1985) found that 6 mg/kg IM resulted in therapeutic serum concentrations lasting for two to five days. • Bush *et al.* (1978) found that the half life for subcutaneously-injected gentamicin in gopher snakes at 24°C was 82 +/– 31 hours, whereas Hodge (1978) reported that the half-life of gentamicin for the broad-banded water snake *Nerodia fasciata* was 48–72 hours at 15°C and 24 hours at 30°C. She speculated that elimination was slower in the terrestrial snake simply because of lower glomerular filtration rates. However, it is also possible that this phenomenon could be accounted for by drug recycling during the retention of urine in the bladder of terrestrial species to allow for water and electrolyte resorption (see comments under carbenicillin). • At present we do not have enough studies available to say whether doses and dose frequencies should generally be higher for freshwater chelonians than for marine or terrestrial species when using renally-eliminated drugs, but this possibility should be borne in mind. • Gentamicin may be more toxic at higher temperatures despite lower plasma levels.
Neomycin	Thorsen (1974) found that enteric Salmonellae were apparently eliminated from terrapins when 200–400 mg of neomycin was added per 5 gallons of tank water at 29.5°C. At lower temperatures, higher doses and longer courses were needed.

Table 16.5 Tetracyclines.

Drug	Characteristics
Doxycycline	In *Testudo hermanni*, 50 mg/kg IM as a loading dose, followed by 25 mg/kg IM every 72 hours was suggested as a therapeutic regime for *Klebsiella* and *Staphylococcus* species (Spörle *et al.* 1991). *Pseudomonas* and *Salmonella* species were considered to be resistant to doxycycline. We have administered doxycycline (50 mg/kg PO every 72 hours) to sick leopard tortoises (*Geochelone pardalis*) with apparent haemoparasitaemia. These animals showed no obvious adverse effects of treatment, recovered clinically and two months later showed a much reduced haemoparasitaemia. Both doxycycline- and chloroquine/primaquine-treated groups responded in similar fashion—there was no negative control group.
Oxytetracycline	• No pharmacokinetic data available for chelonians. • Gauvin (1993) recommended that oxytetracycline be given to desert tortoises (*Gopherus agassizii*) at 5–10 mg/kg every 24 hours IM. • Oxytetracycline may be a good choice for treatment of dermatophilosis (Hermoso de Mendoza *et al.* 1994). • Oxytetracycline has been used in cases of mycoplasmosis 'with some success' (Johnson *et al.* 1998).

Table 16.6 Fluoroquinolones.

Drug	Characteristics
Enrofloxacin	• 21/22 bacterial isolates from chelonian patients at Holly House surgery, United Kingdom, were enrofloxacin sensitive *in vitro*. • We have achieved long-term cure in one subcutaneous mycobacterial abscess with the use of surgery followed by two months of parenteral enrofloxacin at 5 mg/kg every 24 hours. • Based on a study in box turtles (*Terrapene carolina*), Aucoin (1999) suggested a dose of 5 mg/kg IM every four to five days. • For *Testudo hermanni* a dose of enrofloxacin of 10 mg/kg IM every 24 hours was considered appropriate for *Pseudomonas, Klebsiella* and *Salmonella* infections (Spörle *et al.* 1991). • In gopher tortoises (*Gopherus polyphemus*), a dose of 5 mg/kg every 24–48 hours was enough to keep blood levels above MIC for important pathogens (Prezant *et al.* 1994). • In Indian star tortoises (*Geochelone elegans*), a study suggested that enrofloxacin should be given IM at 10 mg/kg every 12 hours for *Pseudomonas* and *Citrobacter* but every 24 hours for other bacteria (Raphael *et al.* 1994).
Ciprofloxacin	In human medicine, ciprofloxacin is generally considered to be more effective than enrofloxacin against *Pseudomonas aeruginosa*. This drug is, however, less well studied in reptiles than enrofloxacin.

Table 16.7 Macrolides.

Drug	Characteristics
Clarithromycin	• May be given orally and is well absorbed even when food is present in the gut. • May achieve high concentrations in the respiratory tract. • Renal and hepatic elimination are both potentially significant. • Effective against *Mycoplasma* in man. • Also effective against *Mycobacteria* (Gunn-Moore *et al.* 1996). • Wimsatt *et al.* (1999) describe the pharmacokinetics of orally-administered clarithromycin in desert tortoises (*Gopherus agassizii*) concluding that 15 mg/kg PO every 2–3 days is the optimum dose to maintain blood levels of 2–7.5 µg/ml. Efficacy in clinical situations has not yet been documented.
Tylosin	Jacobson (1999a) provides an anecdotal report of the use of tylosin at 5 mg/kg IM every 24 hours. Anti-*Mycoplasma* activity may be useful in the treatment of respiratory disease although it may now have been superseded by clarithromycin. Murphy (1973) reported that tylosin at 25 mg/kg every 24 hours IM or PO was safe and effective in the treatment of respiratory infections in reptiles.
Erythromycin	Because it is principally effective against Gram-positive organisms, erythromycin is little used in reptile medicine. Tothill *et al.* (1999) investigated the effect of erythromycin as a prokinetic at 1–2 mg/kg every 24 hours PO on gut passage times in desert tortoises (*Gopherus agassizii*). No significant effect was demonstrated.

Potentiated sulphonamides

Potentiated sulphonamides are:

- broad spectrum;
- potentially bactericidal;
- ineffective against *Pseudomonas*;
- anticoccidial;
- widely distributed within the body;
- not reportedly toxic in any reptiles.

16/22 bacterial isolates from chelonian patients at Holly House surgery, United Kingdom, were trimethoprim/sulphonamide sensitive *in vitro*.

Our current knowledge is based only on anecdotal reports. Jacobson (1999a) and Page & Mautino (1990) both used 30 mg/kg every 24 hours IM for two days then every 48 hours thereafter.

Metronidazole

Metronidazole is an anti-protozoal with efficacy against amoebal trophozoites and extra-intestinal amoebiasis. It is thus effective in treating many symptomatic tortoises. It may not be effective as a sole therapy in eliminating infections, since encysted amoebae may survive unaffected. It is thus less reliable in eliminating the potential threat to other reptiles of carrier chelonians.

Metronidazole is:

- bactericidal;
- metabolised by the liver prior to renal excretion;
- widely distributed within the body;
- able to penetrate abscesses well;
- potentially neurotoxic at high doses.

Stewart (1990) reported that all of his anaerobe isolates from reptiles were sensitive to metronidazole (*Bacteroides, Fusobacterium, Clostridium, and Peptostreptococcus*).

The pharmacokinetic studies of Kolmstetter *et al.* (1997 & 1998) in green iguanas and yellow rat snakes suggest that an appropriate dose for reptiles is 20 mg/kg every other day. It has been suggested that treatment should continue for at least two weeks. Prior to this information, the widely-used anecdotal dose has been 100 mg/kg PO as a single dose, repeated two weeks later.

Klingenberg (1993) notes that prophylactic treatment of Asian box turtles (*Cuora* spp.) with metronidazole at transportation 'dramatically improves survival rates'.

Dimetridazole

Jacobson *et al.* (1983) successfully medicated several hundred red-footed tortoises (*Geochelone carbonaria*) suffering from amoebiasis by soaking every litre of food (dry dog food, fruits and vegetables) in 300 ml of dimetridazole solution made up with 2.6 ml Emtryl® (Salisbury Laboratories, Iowa) per litre of water.

Drug combinations

This approach is particularly useful in situations where culture and sensitivity data are not available or where mixed infections are present (a common occurrence: 8/21 isolates from sick chelonians at Holly House surgery, United Kingdom, involved more than one possible pathogen). It may also retard the emergence of resistance (Table 16.8).

Topical antibacterials

Topical medications may be applied to the skin, shell, mouth, nose, eyes, wounds and burn sites. The normal flora of chelonian skin and oral cavity is rich in members of the Enterobacteriaceae and various fungi. It is primarily these opportunist pathogens which are responsible for skin disease and which colonise the epithelia of animals with compromised defences. Similarities exist with burn management in man, where initial Gram-positive infections are superseded by Gram-negative opportunists that may precipitate life-threatening bacteraemia/septicaemia. For example:

- Jackson & Fulton (1970) added to the observations of Kaplan (1957) upon septicaemic cutaneous ulcerative disease (SCUD) in freshwater turtles. In conjunction with positive blood cultures these animals consistently yielded *Citrobacter* and *Serratia* from their skin lesions. *Proteus, Pseudomonas* and *Aeromonas* were present in lower numbers.
- Ladyman *et al.* (1998) described an outbreak of necrotising dermatitis in hatchling western swamp tortoises (*Pseudemydura umbrina*) associated with an unidentified *Pseudomonas* sp.

When treating skin disease, the surface of any lesion is likely to be heavily colonised by non-pathogenic organisms. When the shell is involved, consider culture from a biopsy sample taken using a bone biopsy tool. The whole biopsy sample minus the most superficial layer should be implanted into transport medium before submission for aerobic and anaerobic culture. The true pathogen(s) is more likely to be isolated from below the surface.

It is often helpful to maintain aquatic chelonians in dry-dock for a day or two whilst topical preparations are applied. Alternatively, waterproof but 'breathable' liquid plastic dressings such as Germolene New Skin® (SmithKline Beecham) have been used to prevent contamination of open wounds in aquatic mammals (Lucas *et al.* 1999). Maintenance of water hygiene is of the utmost importance.

Table 16.9 summarises the topical preparations.

Table 16.8 Some antibacterial drug combinations.

Drug combination	Discussion
Gentamicin or amikacin plus piperacillin or carbenicillin	The relationship between gentamicin and carbenicillin is a complicated one. Lawrence (1983) reviews the literature on this subject citing material which suggests that combination therapy is clinically effective although the two antibiotics may chemically inactivate each other and should be injected into separate sites. Amikacin may not be prone to the same problem. Jacobson (1999a) describes the use of a protocol where the dose of gentamicin or amikacin is given every third day, followed on each occasion by carbenicillin or piperacillin 48 hours later.
Gentamicin or amikacin plus ceftazidime	Jacobson (1999a) suggests that the aminoglycoside be given on day one followed by ceftazidime on day two. Repeat doses of both drugs are given every third day maintaining the same interval between the two up to a total of 7–9 aminoglycoside and 21 ceftazidime doses. No justification for this protocol is given—it is not clear, at least to this author (RW), why the ceftazidime should not be given on day one, along with the aminoglycoside, or whether there is a firm basis for a limit of 21 ceftazidime doses. Mayer & Nagy (1999) investigated the synergistic effects of aminoglycosides (amikacin and netilmicin) in combination with third-generation cephalosporins (cefoperazone, ceftriaxone, and ceftazidime) against 18 clinical human isolates of *Pseudomonas* spp. Using a disc-diffusion method it was demonstrated that amikacin or netilmicin in combination with one of the three cephalosporins exhibited synergy against 7–12/18 isolates.
Enrofloxacin plus ceftazidime	We have used this combination in many sick chelonians. Both drugs are relatively safe and have many advantages that are discussed above. We have experienced no problems when both are given simultaneously at standard dose rates (although we have not physically mixed the two preparations together). Mayer & Nagy (1999) found that, when a quinolone (ciprofloxacin, ofloxacin or pefloxacin) was used in combination with a third-generation cephalosporin (cefoperazone, ceftriaxone or ceftazidime) against 18 clinical human isolates of *Pseudomonas* spp., synergistic effects were seen against 3–5/18 isolates. No antagonism was found with these combinations.
Aminoglycoside plus metronidazole	The combination of amikacin and metronidazole has been mentioned by Klingenberg (1996). The addition of metronidazole, which is effective against anaerobes, complements the spectrum of the aminoglycoside that is not. However, no concrete pharmacological data is available for metronidazole and other agents with anti-anaerobe activity, such as ceftazidime.

Table 16.9 Topical antibacterials.

Drug	Discussion
Chloramphenicol	Jackson & Fulton (1970) reported some success in controlling mortality from septicaemic cutaneous ulcerative disease (SCUD) of turtles with the addition of chloramphenicol (250 mg/20 gallons water) to the tank water. The patients were likely to have been taking on some drug orally as well as being exposed topically.
Gentamicin or neomycin (e.g. Cidomycin®, Hoechst)	• Inactivation by inorganic debris and poor penetration into tissue are potential problems for topical aminoglycoside preparations. • Sensitivity of Gram-negatives to these agents is variable. • Potential for systemic toxicity exists if used on ulcerated surfaces.
Polymixin B/bacitracin (e.g. Polyfax®, Dominion, UK)	The possibility that absorption may occur should be considered since these drugs are potentially nephrotoxic. They are also inactivated by organic debris and penetrate tissue relatively poorly.
Mafenide acetate	• Has been widely used in the management of burns in human medicine as a 5%–8.5% solution. • It is bacteriostatic, active in the face of pus and broad spectrum with efficacy against Enterobacteriaceae including *Pseudomonas* spp. • In one study (in rats) efficacy in controlling burn pathogens was superior to that of silver sulphadiazine (Gray *et al.* 1991). • Systemic toxicity is a potential problem. We have no experience of its use in chelonians.
Fusidic acid (Fucidin®, Leo)	• Activity restricted to Gram-positive bacteria. • Probably a poor choice for most chelonian patients unless dealing with a known staphylococcal or streptococcal problem. • Penetration of tissue is superior to that of topical aminoglycosides.
Mupirocin (Bactroban®, SmithKlineBeecham)	• Activity is largely restricted to Gram-positives. • May have some anti-fungal activity. • Good penetration of granulomas and organic debris. • Has a unique mechanism of action and no equivalent antibiotic available for systemic use. Hence, cross-resistance should not be a problem. • Care should be taken when applying Bactroban to ulcerated surfaces or mucous membranes, since the polyethylene glycol vehicle is potentially nephrotoxic.
Silver sulphadiazine (SSD) (Flamazine®, Smith & Nephew):	SSD is broad spectrum, non-stinging, non-staining, not readily susceptible to bacterial resistance and active in the face of organic debris. It is thus ideally suited to the management of many chelonian skin problems, wounds or burns. Hadjiiski & Lesseva (1999) compared the activity of fusidic acid, chlorhexidine, iodines, mupirocin, SSD and SSD plus cerium in the management of burn wounds in man. They concluded that SSD and SSD/cerium were the most effective.
Silver sulphadiazine plus cerium (Flamacerium®)	The addition of 2.2% of the rare earth metal cerium salt to SSD causes the formation of a relatively hard, yellow, leather-like eschar on the lesion, with excellent resistance to infection and good long-term adherence to wounds.
Iodines	• Virucidal, fungicidal, and bactericidal. • Tamed® iodines have reduced irritancy although the active ingredient remains elemental iodine. • Povidone-iodine (Betadine®, Seton) is non-stinging and active in the face of pus and organic debris. • Polyhydroxydine 1% (Xenodine®) may be superior to povidone-iodine against Gram-positive and Gram-negative bacteria.
Chlorhexidine	As a 0.5%–2% solution, chlorhexidine has many advantages: it is non-toxic (at least in mammals), non-irritant, not inactivated by organic matter, persistent for up to 48 hours after application and possessing activity against bacteria, fungi and many viruses. However, activity against Enterobacteriaceae is variable. It is effective against *E. coli* (Oie *et al.* 1999) but resistance develops in *Pseudomonas* (Lloyd & Lamport 1999; Tattawasart *et al.* 1999) and *Serratia*.
Triclosan	Triclosan is less effective (as a 1% solution) against Gram-negative bacteria than 4% chlorhexidine gluconate (Faoagali *et al.* 1999).
Honey	Al-Waili & Saloom (1999) found that honey plus systemic antibiotics was a superior treatment to ethanol/povidone-iodine plus systemic antibiotics in treatment of Gram-positive/Gram-negative laparotomy wound infections in man.
Malachite green	Leong *et al.* (1980) found that immersing hatchling sea turtles in seawater containing 0.1–0.4 ppm malachite green caused no obvious external injury. Irritant or toxic effects were seen at 0.8 ppm. Jacobson *et al.* (1980) found that the lesions of cutaneous mucormycosis in Florida soft-shell turtles (*Trionyx ferox*) regressed when the animals were immersed for 15 minutes in 0.15 mg/l malachite green three times daily for one week. Conjunctivitis was a side effect and the animals were rinsed in fresh water to reduce this problem.

Johnson *et al.* (1998) describe a method for application of antibiotic drops to the nasal cavity of tortoises suffering from rhinitis. The animal is tipped onto its back and the medication is applied to the choana. When pressure is applied to the intermandibular area the drugs are flushed in a retrograde fashion through the nasal cavity.

Choice of antibacterial

While the best way to choose an antibacterial is after culture and sensitivity testing, Table 16.10 summarises the agents commonly isolated from various sites and the drugs most likely to be effective in their treatment.

ANTIFUNGALS

The principles of antifungal therapy are much the same as for bacterial disease. Culture and sensitivity plus cytological or histological evidence of pathogenicity are important. Localised subcutaneous or intrapulmonary granulomatous lesions should be surgically excised prior to drug therapy if possible.

Fungi isolated from mycotic lesions are opportunists from the surrounding environment or present as members of the normal flora of chelonian skin or gastrointestinal tract. The yeasts most commonly isolated include *Candida* (32/56 isolates), *Torulopsis* (9/56), *Rhodotorula* and *Trichosporon* (Kostka *et al.* 1997). Yeast infections are commoner in herbivorous chelonians. *Aspergillus, Fusarium, Geotrichum, Paecilomyces, Candida* and various Phycomycetes (now an obsolete classification: superseded by Zygomyces) were the fungi most commonly identified as pathogens by Migaki *et al.* (1984).

Mycoses can be divided into:

- superficial mycoses (e.g. oral or enteric candidiasis);
- subcutaneous (intermediate) mycoses (e.g. zygomycosis involving *Mucor* or *Absidia*);
- systemic mycoses (e.g. aspergillosis, trichosporonosis, paecilomycosis).

Superficial mycoses may be treated topically and/or systemically whilst subcutaneous and systemic mycoses require systemic therapy.

Superficial mycoses

Gastrointestinal mycoses

Candida albicans can be isolated from the faeces of healthy tortoises: the pathogenic role of these yeasts remains to be proven.

Zwart & Buitelaar (1980) described three cases in which *Candida tropicalis* was isolated from the faeces of sick tortoises. In two of these, oral natamycin 3 mg/kg led to a decrease in yeast numbers and one animal recovered although the other subsequently died. It is unknown whether these animals had concurrent disease processes. Natamycin is believed not to be absorbed from the gut.

Jacobson (1980) gives a dose for nystatin of 100,000 IU/kg every 24 hours PO for the treatment of oral or gastrointestinal candidiasis.

We have seen several diarrhoeic Mediterranean *Testudo* spp. with abundant yeasts, subsequently identified in culture as *Candida albicans* or *C. guilliermondii*, present in faecal wet mounts. Some of these had other concurrent problems but at least one recovered after treatment with oral ketoconazole 20 mg/kg every 24 hours plus supportive care. In man, fluconazole has become the treatment of choice for candidiasis of the mucosae.

Systemic/subcutaneous mycoses

Page *et al.* (1991) describe the pharmacokinetics of orally administered ketoconazole in desert tortoises (*Gopherus agassizii*). Ketoconazole (Nizoral®, Janssen) has subsequently become the most widely used anti-fungal in chelonian medicine. However, it may soon be superseded by triazoles, such as itraconazole and fluconazole, which have a broader spectrum of activity, fewer side effects (at least in mammals) and are better absorbed than ketoconazole. Ketoconazole is available only as oral preparations whereas fluconazole is also available in injectable form (Diflucan infusion®, Pfizer).

The spectrum of activity of itraconazole includes *Candida, Malassezia* and *Aspergillus*. Fluconazole has a similar spectrum but also penetrates the central nervous system and saliva. It is the treatment of choice for oral and urinary tract candidiasis in man. However, Cabanes *et al.* (1997) reported a case of cutaneous hyalohyphomycosis in a captive loggerhead sea turtle (*Caretta caretta*) from which a strain of *Fusarium solani* was isolated that proved resistant to 5-fluorocytosine, fluconazole, itraconazole and ketoconazole.

The only pharmacokinetic data for itraconazole in reptiles stems from the work of Gamble *et al.* (1997). They used an oral dose of 23.5 mg/kg once daily in spiny lizards to achieve a steady state in blood and liver levels above reported MICs for common fungal pathogens. Whitaker & Krum (1999) used fluconazole and enrofloxacin together for two to three months in the treatment of sea turtles with 'safety and efficacy'.

Amphotericin B has been given intracoelomically at 1 mg/kg every 24 hours for two to four weeks (Rosskopf quoted in Mader 1996). This drug is potentially nephrotoxic.

Respiratory mycoses

An alternative approach to systemic therapy is intrapneumonic drug delivery via an indwelling catheter secured in a hole drilled through the carapace (Divers 1998b). This is a useful technique for delivery of potentially toxic antifungals such as amphotericin B. Further details, including drugs used and their dosages, are given in Table 16.2.

Topical antifungals

Chlorhexidine and iodines are discussed under Antibacterials but also have antifungal activity. Both appear to be suitable for external and oral use. We have used povidone-iodine solutions sparingly without obvious adverse effect in numerous cases of stomatitis, where both yeasts and bacteria were evident on cytological preparations.

A variety of topical azole preparations are available for use in man (clotrimazole, miconazole, econazole, tioconazole). No objective data is available on their use in reptiles.

Table 16.10. Suggested antibiotics for various clinical presentations (format after Jacobson 1993).

Site	Diagnosis	Agents commonly isolated	Suggested drugs
Skin/shell	dermatitis, wounds	*Pseudomonas* *Proteus* *Klebsiella* *Morganella* *Citrobacter* *Providencia* *Aeromonas* (aquatic spp.)	Systemic: ceftazidime enrofloxacin amikacin alone or in combination
			Topical: silver sulphadiazine (SSD) SSD plus cerium iodines
		Dermatophilus	ampicillin oxytetracycline
Subcutaneous	abscess granuloma pyogranuloma	*Mycobacteria*	enrofloxacin clarithromycin alone or in combination
		Enterobacteriaceae	As for skin/shell
		anaerobes	metronidazole ceftazidime
		Fungi	ketoconazole, itraconazole
Oral cavity	stomatitis	*Pseudomonas* *Aeromonas*	ceftazidime enrofloxacin amikacin alone or in combination
		Candida	ketoconazole
Respiratory tract	pneumonia	*Pseudomonas* *Morganella* Other Enterobacteriaceae	ceftazidime enrofloxacin amikacin alone or in combination
		Aspergillus *Paecilomyces*	ketoconazole, itraconazole
	rhinitis	*Mycoplasma* *Pasteurella*	enrofloxacin clarithromycin
Skeletal	osteomyelitis septic arthritis	Enterobacteriaceae	ceftazidime enrofloxacin amikacin alone or in combination consider the use of impregnated PMMA beads
Ear	otitis media	Enterobacteriaceae *Staphylococci* *Streptococci*	ceftazidime enrofloxacin amikacin alone or in combination
Septicaemia		Enterobacteriaceae	ceftazidime enrofloxacin amikacin alone or in combination
Gastrointestinal		Candida	ketoconazole

ANTIVIRALS

Marschang *et al.* (1997) demonstrated that both acyclovir (at 50 µg/ml) and ganciclovir (at 25 µg/ml and 50 µg/ml) reduced chelonian herpesvirus replication *in vitro*.

The clinical use of acyclovir in the treatment of apparent herpes-virus infection is based upon anecdotal information (e.g. Klingenberg 1996). The drug has been administered both topically (e.g. Cooper *et al.* 1988) for stomatitis cases and orally, by stomach tube or by oesophagostomy tube, in patients with upper respiratory tract disease.

We have administered the oral preparation to large numbers of tortoises at 80 mg/kg/day and 80 mg/kg every eight hours for periods of up to two months (often in combination with antibiotics and/or antifungals) without observing adverse effects. Subjectively, survival rate in herpesvirus infection appears to be improved when acyclovir is used three times daily. Results with once-daily dosing have been disappointing. In a group of *Testudo horsfieldi* with upper respiratory tract disease, from which herpesvirus was isolated, acyclovir-treated individuals appeared to derive no benefit above that of a control group (McArthur 2000).

The amino acid arginine is important in herpes-viral replication. In human medicine, low levels of dietary arginine and increased levels of lysine administered PO appear to reduce the frequency of recurrence of signs of herpes simplex (Griffith *et al.* 1978). In veterinary medicine, oral lysine has been anecdotally reported to be of benefit in the management of relapsing feline herpes keratitis (given at 100 mg/cat every 12–24 hours). This apparently-safe therapeutic option should perhaps be considered in the management of chelonians at risk of herpes-virus-induced disease.

Severely affected patients suffering from viral disease should generally be treated with antibiotics and possibly antifungals to reduce the risk of complications due to secondary pathogens. There are many accounts in the literature (e.g. Oettle *et al.* 1990) of mortality in viral disease apparently due to septicaemia/bacteraemia.

PARASITICIDES

Not all parasites are pathogenic. Chelonians are hosts to a variety of protozoan and metazoan organisms that cause minimal if any problem. Amoebae, other than *Entamoeba*, which are frequently present in the gut, have not been recorded as being pathogenic to chelonians. Enteric ciliates are probably benign commensals. The situation with regard to enteric flagellates is less clear, but low numbers may be normal. Oxyurid infections are generally asymptomatic although their subclinical impact is a matter of debate.

Consideration of the parasite's life cycle is fundamental (Table 16.11) to management. Those with direct life cycles are more difficult to control in captive animals, since auto-infection can occur. In this situation hygiene is important. Parasites with indirect life cycles can be eliminated when an effective parasiticide is administered and access to the intermediate host is prevented. Carnivorous species may be protected from re-infestation by freezing their prey for 30 days before thawing and feeding.

Table 16.11 Direct and indirect life cycles in chelonian parasites.

Parasites with a direct life-cycle	Parasites with an indirect life-cycle
coccidia	some ascarids
amoebae	acanthocephalans
Cryptosporidium	cestodes
flagellates	digenetic trematodes
some ascarids	
Proatractis	
oxyurids	
monogenetic trematodes	

Macrocyclic lactones

Ivermectin is known to be toxic to many chelonians and should be avoided. Since life-threatening metazoan parasite infestations are, in our experience, rare in chelonians there seems little current justification for risk-taking (Teare & Bush 1983; Bodri *et al.* 1993).

There are species differences in susceptibility to ivermectin toxicosis. Box turtles (*Terrapene* spp.) appear to be relatively resistant, whilst red-eared sliders (*Trachemys scripta*) and most *Testudo* spp. are often (but not always) severely affected.

Macrocyclic lactones such as milbemycin (Interceptor®, Novartis) or moxidectin (Cydectin®, Fort Dodge), with reduced potential for central nervous system toxicity in mammals, may be safer for chelonians than ivermectin. Milbemycin has been administered to box turtles (*Terrapene carolina major*) and red-eared sliders without apparent adverse effect (Bodri *et al.* 1993). Doses of up to 1 mg/kg were given orally and up to 0.5 mg/kg by subcutaneous injection. Evidence of nematodicidal effect was recorded although this was not exhaustively investigated. Other than levamisole, which is little used these days, milbemycin appears to be the only available injectable agent effective against nematodes—a potential advantage in aggressive species.

Sulpha drugs

Various sulpha drugs have been used to treat coccidiosis in reptiles. Klingenberg (1995) suggests that sulphadimethoxine (50 mg/kg PO every 24 hours for 5–7 days and then every other day as needed) is the drug of choice for coccidiosis. In the United Kingdom, this drug is available as Coxi Plus® (Vetrepharm). Trimethoprim/sulphamethoxazole and trimethoprim/sulphadiazine are the alternatives.

Benzimidazoles

The benzimidazole spectrum of activity includes ascarids (and larval stages), oxyurids and some cestodes (Table 16.12). Fenbendazole, oxfendazole, albendazole, mebendazole and thiabendazole have been used in reptiles (Mader 1996). In mammals, fenbendazole is metabolised to oxfendazole. The capacity for this conversion in chelonians is unknown. It has been speculated that administration of oxfendazole may be preferable.

Table 16.12 Benzimidazoles.

Drug	Use
Thiabendazole	Thiabendazole has been used at 50–400 mg/kg by various authors (reviewed by Holt 1981). However, even doses of 400 mg/kg were apparently ineffective in eliminating *Angusticaecum* ascarids from chelonians (Holt 1978; Holt *et al.* 1979).
Oxfendazole	We have administered this drug (in the form of Systamex®, Schering Plough, 65 mg/kg) orally to numerous tortoises without adverse effect.
Fenbendazole	Holt (1981) found this drug to be 'safe and effective in eliminating tortoise ascarids and oxyurids' at 50 mg/kg given twice with a three-week interval. At 100 mg/kg it is also effective against oxyurid infestations when administered per cloaca (Innis 1995). This author suggested that at least some orally administered anthelminthics would be absorbed before reaching parasites residing in the large intestine. When these parasites do not feed on the mucosa, as is the case for oxyurids, they remain unaffected. This explanation was offered for continued presence of adults and ova in faeces after oral fenbendazole administration on two occasions. When the same three individual tortoises were treated once per-cloaca shedding ceased. The possibility that this apparent cure was the cumulative result of oral and then subsequent cloacal worming cannot be excluded from the data presented. This technique has obvious advantages when dosing intractable animals, which are difficult to stomach tube.

Piperazine

Piperazine use in reptiles has been associated with undesirable side effects (Jackson 1974) and is probably best avoided in chelonians. The citrate component of piperazine citrate may be potentially toxic in its own right, as it may precipitate hypocalcaemia (Soifer 1978).

Levamisole

Zwart & Ham (1972) recommended the use of 50 mg/kg PO in the treatment of chelonian oxyurids. Frank (1976), however, found that up to 300 mg/kg might be necessary, whilst Moser (1973) concluded that efficacy against ascarids and oxyurids was poor.

Praziquantel

Praziquantel is well-tolerated and effective against cestodes and trematodes (Frank & Reichel 1977). These authors do not specifically mention the treatment of chelonians but rather reptiles as a whole. Higher doses are required for the treatment of pseudophyllidean cestodes (30 mg/kg) than for other species (5 mg/kg).

The importance of spirorchidiasis in sea-turtle medicine has lead to increased interest in trematodicides. Recently, Jacobson *et al.* (2002) investigated the pharmacokinetics of praziquantel in loggerhead turtles (*Caretta caretta*). The conclusion of this pilot study was that, in this species, orally-administered praziquantel should be given three times at 25 mg/kg with three hours between doses. Clinical efficacy was not demonstrated in this study but has previously been reported by Adnyana *et al.* (1997) for praziquantel in green turtles (*Chelonia mydas*).

Parasitic diseases

Coccidiosis

A syndrome of extra-intestinal coccidial infestation, characterised by high mortality, has been described in a small number of chelonian patients by Jacobson *et al.* (1994) and Garner *et al.* (1998). The exact identity of the parasite(s) involved is unknown and the disease is generally referred to as intranuclear coccidiosis since developmental stages are found in this location in the cells of a wide variety of internal organs. Anecdotally, toltrazuril (Baycox®, Bayer) has been used to treat suspected cases.

Cryptosporidiosis

Treatment of cryptosporidiosis is problematic. Trimethoprim/sulphonamide combinations have been suggested but, in practice, have been disappointing in a variety of species. Recently, Lefay *et al.* (2001) reported apparent success in reducing parasite excretion (although not mortality in this pilot study) by using halofuginone lactate (Halocur®, Intervet)—a synthetic quinazolinone derivative—in calves.

Amoebiasis

Metronidazole and dimetridazole have proved effective against extra-intestinal disease. Chloroquine may also be suitable for this purpose. Pharmacokinetic data is now available for the former (see Formulary and above).

Elimination of enteric infection is more problematic. Asymptomatic chelonians continuing to shed cysts are a threat to other reptiles in a collection. Iodoquinol (50 mg/kg PO sid for 3 weeks) has been found to be effective in preliminary trials.

Trypanosomiasis

Telford (1984) suggested that 'in view of the usually low parasitaemias found, prognosis would appear to be excellent without treatment'.

Haemogregarines

No information is available, but antimalarials (such as doxycycline, primaquine or chloroquine) or anticoccidials (such as sulphadimidine or sulphamethoxazole) might be effective.

Malaria

(See comments concerning the existence (or not) of *Plasmodium* infections in chelonians in the Clinical Pathology section of this book.) Chloroquine, primaquine and doxycycline are potential options. Scorza (1971) improved survival in malarial lizards

Table 16.13 Suggested parasiticides for specific indications in chelonians.

Parasite	Suggested treatment
Coccidia	Sulphadimethoxine, trimethoprim/sulphamethoxazole or trimethoprim/sulphadiazine (may be ineffective for *Caryospora*). Toltrazuril for intranuclear coccidiosis.
Cryptosporidium	Unknown, possibly paromomycin or halofuginone lactate (Lefay *et al.* 2001).
Entamoeba	Oral metronidazole or dimetridazole for clinical disease. Possibly iodoquinol to eliminate shedding.
Flagellates	Oral metronidazole or dimetridazole.
Ascarids	Oral or cloacal fenbendazole, oxfendazole.
Oxyurids	Oral or cloacal fenbendazole, oxfendazole, thiabendazole.
Proatractis	Oral fenbendazole, piperazine ineffective. Systemic ivermectin inconclusive (Rideout *et al.* 1987). Hygiene is likely to be an important consideration. Milbemycin is a further option.
Cestodes	Oral or systemic praziquantel (Frank & Reichel 1977). Alternatively, dichlorophen, niclosamide, or bunamidine have been used (reviewed by Holt 1981).
Trematodes	Oral or systemic praziquantel (Frank & Reichel 1977).
Cistudinomyia	Manual removal.
Ticks	Manual removal, environmental decontamination.
Cloacal mites	Milbemycin?

two-fold by the intramuscular injection of 0.15 ml of an iron-dextran complex containing 50 mg/ml iron.

Haemoproteus

Telford (1984) stated that no data was available on treatment. Doxycycline, chloroquine and primaquine are possible options. Pharmacokinetic data exists for doxycycline in chelonians.

We have administered chloroquine (5 mg/kg PO once weekly) and primaquine (0.5 mg/kg PO once weekly) in combination to sick leopard tortoises (*Geochelone pardalis*) with apparent haemoparasitaemia (*Sauroplasma*-like). No immediate adverse effects were observed. Some recovered clinically whilst others died (although not during the treatment period). Two months later the surviving animals showed a much-reduced haemoparasitaemia. Both doxycycline- and chloroquine/primaquine-treated groups responded in similar fashion—there was no negative control group.

Summary

Table 16.13 pairs the parasite with the most effective parasiticide.

FLUID THERAPY

All fluids should be warmed to PBT before administration.

Determining hydration status

The ability to quantify fluid deficits is important. The potential for fluid overload may exist when fluids are administered too rapidly by non-oral routes. Patients with renal, respiratory or cardiac dysfunction are particularly at risk of developing oedema, although even relatively healthy animals are susceptible. Cardiac disease is probably rare, but renal disease is not, and peripheral oedema is a common sign in these patients even without fluid therapy.

In practice, an assessment of hydration is not easily made. It is frequently stressed in published texts that hydration status should be assessed, corrected and maintained, but recommendations as to the details of how this be achieved are speculative. A special problem arises in chelonians that use their urinary bladder as a water reservoir. In these animals, the first change occurring with the onset of water deprivation is the shift of water from the bladder into other body compartments. Changes in blood parameters and clinical signs of dehydration will certainly be reduced in magnitude (possibly even completely negated) by this shift until bladder water is exhausted. Only at this point does the animal begin to suffer adverse effects of dehydration.

History of weight change

A precise knowledge of weight loss is an invaluable aid in the quantification of dehydration, particularly in the acute case. This will never be an entirely accurate measure, since variables such as loss of gut content, fat reserves or body protein, oviposition, urination and tissue oedema must be taken into account. Many chelonian owners/keepers are highly motivated and can readily be persuaded to weigh their pets accurately every couple of weeks.

Clinical signs

Both Klingenberg (1996) and Bennett (1998) suggest that, in reptiles, hydration status can be ascertained in much the same manner as in mammals. They state that mild (5%–8%) dehydration is characterised by loss of skin elasticity and skin 'wrinkling'. With further deterioration, the eyes appear sunken and the mucous membranes become dry and tacky. Some caution must be exercised since healthy reptiles have drier mucous membranes than mammals. Sunken eyes and loss of skin elasticity may also result from cachexia.

Haematocrit

An above-normal PCV is consistent with dehydration. However, variables such as species, sex (males have higher PCV in some species) and season must be taken into consideration. Anaemia is probably common in chronically-ill reptiles and may mask an elevation in PCV.

Blood biochemistry

As with PCV, above-normal blood albumin is consistent with dehydration, but hypoproteinaemia is also common in sick chelonians and may mask the effects of dehydration. An added complication is the phenomenon of elevated albumin during the breeding season in females of many species.

Klingenberg (1996) states that dehydration should be considered a potential cause of elevation in blood uric acid. However, the work of Dantzler & Schmidt-Nielson (1986) suggests that glomerular filtration rates, and hence blood uric acid levels, in terrestrial chelonians may actually be quite resistant to water deprivation.

Our experience suggests that elevated blood uric acid levels (>1,000 µmol/l) are likely to indicate renal insufficiency. There may or may not be concurrent fluid/electrolyte imbalance.

Blood urea has been widely discounted as a useful indicator in reptilian biochemistry. This may not be true for groups, such as terrestrial chelonians, where urine is retained in the bladder as a fluid reserve against periods of water deprivation. Urea readily crosses biological membranes and may equilibrate between bladder urine and blood – in contrast to uric acid, which is precipitated in the bladder as insoluble urates. At Holly House surgery, United Kingdom, hyperuricaemic patients are almost invariably also hyperuraemic. A further subset of cases, however, is normouricaemic but hyperuraemic. Many of this group seem likely, from history and physical examination, to be dehydrated. This theory is supported by the observation that healthy hibernating *Testudo hermanni* become hyperuraemic towards the end of hibernation (Gilles-Baillien & Schoffeniels 1965).

A normal blood uric acid level in conjunction with elevated urea may indicate dehydration with normal renal function.

Tear gland secretion

Prange & Greenwald (1980) demonstrated that the tear gland secretion of green turtles (*Chelonia mydas*) varies in composition with hydration status (Table 16.14).

Terrestrial reptiles may also produce salt-rich secretions from their noses that may vary in composition with hydration status (Schmidt-Nielson *et al.* 1963). However, we are unaware of any studies relating directly to terrestrial chelonians.

Table 16.14 Tear composition in green turtles under differing degrees of hydration (Prange & Greenwald1980).

Hydration status	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)
Normal	388	26	401
Dehydrated 10–20 days out of water	834	28	1020
Rehydrated in fresh water	215	38	222

Whole blood and haemoglobin

Indications for the use of whole blood are not common in chelonians but include acute haemorrhage and life-threatening anaemia of other origin. Rosskopf (2000) reports the use of desert tortoise (*Gopherus agassizii*) blood to transfuse 'several species of smaller chelonian with apparent clinical success.' Divers (1997c) described transfusion of blood from Hermann's tortoises into a leopard tortoise 'without obvious ill effect'. McCracken *et al.* (1994) described the administration of blood transfusions to a snake (with haemolytic anaemia) and a lizard (with nutritional anaemia).

Various authors have suggested that cross-matching may be advisable but have not described the practicalities thereof. No specific cases have yet been described in the literature in which adverse effects were detected. Reptiles as a group do not appear to be very susceptible to anaphylactic reactions. However, the clinicopathological tools by which adverse reactions might be detected, particularly in the longer term, are still undeveloped.

The signs of volume overload, although of very real significance, may be difficult to recognise in chelonians. Although we know that viral disease is common and potentially fatal in many species of chelonian, we currently lack the technology to screen donors. In summary, transfusion techniques are in their infancy in chelonians and should be employed with caution. It may be difficult to justify endangering the health of potential donors in view of the poorly defined benefits and potential risks to recipients given our current state of knowledge.

The possibility has recently arisen of administering purified bovine haemoglobin (Oxyglobin®, Biopure). This product is a potent colloid with oxygen-carrying capacity. In a pilot study, Wimsatt (2001) administered doses of 20 ml/kg via a cardiac access port into the ventricle at a rate of 99 ml/hr to healthy desert tortoises (*Gopherus agassizii*). No serious sequelae were observed. Discoloured mucous membranes are a normal finding in patients after administration of oxyglobin. In mammals, volume overload has proved a potential danger.

Fluids for oral (or colonic) rehydration

The oral route is an excellent method of rehydration if vomiting or regurgitation does not occur, and if the patient can be stomach tubed or has an oesophagostomy tube fitted. Potassium deficits can be safely replaced. In man, the formulation most likely to promote uptake of salt and water may be equimolar sodium chloride and glucose solution (100 mmol/l of each). However, this is aimed at replacing mixed fluid and electrolyte losses (i.e. isotonic dehydration) incurred through, for example, diarrhoea (Michell *et al.* 1989).

The ideal solution for many sick chelonians is unknown—no objective data are available. The ideal glucose content is also unknown. In theory, increasing glucose content has advantages, since hypoglycaemia can be alleviated and hepatic lipidosis avoided. However, a risk of inducing hypernatraemia through osmotic movement of water into the gut also exists. The influence of such therapy on gut flora is unknown.

At Holly House surgery we routinely administer water and liquidised vegetables, which will contain sugars, with apparent success.

Table 16.15 Blood osmotic concentration in various chelonian species.

Species	Blood osmotic concentration (mOsm/l)	Circumstances	Reference
Testudo hermanni	258–449	Normal seasonal variation	Gilles-Baillien & Schoffeniels (1965)
Gopherus agassizii	291–334	Wild animals, summer	Minnich (1976)
	249–346	Wild animals, winter	Christopher *et al.* (1999)
	247–314	Wild animals, spring	
	252–352	Wild animals, summer	
	250–346	Wild animals, autumn	
Caretta caretta	465	Sea water	Schoffeniels & Tercafs (1965)
	300–343	Normal seasonal variation in wild adults	Lutz & Dunbar-Cooper (1987)
	224	After three years in fresh water	Schoffeniels & Tercafs (1965)
Chelonia mydas juveniles	270	Sea water	Holmes & McBean (1964)
	207	After two months in fresh water	Holmes & McBean (1964)
Chelydra serpentina	315		Semple *et al.* (1969)
Trionyx spiniferus	280	Fresh water	Dunson & Weymouth (1965)
Trachemys scripta	260		Hutton & Goodnight (1957)

Fluids for parenteral administration

Osmolality

There has been some debate in the past about the ideal osmolality of fluids for intravenous/intracoelomic infusion in chelonians. The data presented in Table 16.15 below and in the chapter on Clinical Pathology suggest that at least terrestrial and marine chelonians can tolerate a wide range of plasma osmolalities without adverse effect. The data for *Testudo hermanni* and *Caretta caretta* is very much more complete than for the other species listed, and demonstrates the pronounced seasonal variation that probably occurs in most temperate species.

It may be appropriate to take advantage of solutions isotonic to mammalian blood (these solutions generally have total osmolality in the region 280–310 mOsm/l). The osmolality of blood in a given patient can be approximated using the equation (Michell *et al.* 1989):

Osmolality = 2 (Na + K) + glucose + urea (all measured in mmol/l)

Table 16.16 gives pre-treatment blood osmotic concentration for some representative patients at Holly House calculated using this equation.

The comparison between this data and that for healthy *T. hermanni* (see Clinical Pathology chapter) is not easy to interpret, since our patients include some animals which did not hibernate and others that did, but which seem likely to have emerged earlier than those of Gilles-Baillien & Schoffeniels (1965). It appears, however, that the tendency in this small series is for our chronically-sick animals to be isotonic or hypotonic to non-hibernating, healthy chelonians. Even the animal with stomatitis, which might be expected to have trouble in drinking, had no evidence of hypertonicity.

Table 16.16 Blood osmolality in representative clinical cases.

Species	Month	Condition	Approximate blood osmolality
Furculachelys whitei	March	URTI.	295
Testudo graeca	April	stomatitis	291
Testudo sp.	March	ovarian stasis	291
Testudo hermanni	June	chronic ill-thrift	227
Testudo sp.	March	rhinitis	296
Testudo graeca	March	chronic ill-thrift	300
Geochelone pardalis	April	viraemia	277

If fluids are to be administered, it seems reasonable that they should include a significant amount of sodium. However, hypernatraemia actually appears to be uncommon in our patients (our maximum of 163 mmol/l for a patient at Holly House surgery is lower than the maximum recorded in healthy animals post-hibernation of Gilles-Baillien & Schoffeniels 1965).

It has been suggested (Prezant & Jarchow 1997) that parenteral administration of hypotonic solutions is preferable because (a) reptiles have a higher proportion of body water in the intracellular compartment than mammals, and (b) because reptiles commonly suffer from 'hypertonic dehydration' (water deficit without electrolyte deficit). To this author, these appear to be questionable arguments.

The belief that chelonians have a greater proportion of total body water in the intracellular fraction than mammals stems from Thorson (1968a & 1968b). Intracellular fluid (ICF) volume was calculated by subtracting extracellular fluid volume from total body water but did not take into account water held in the

bladder. Thorson himself admitted that this might have lead to an overestimation of ICF volume.

The case for believing that hypertonic dehydration is common in chelonians has not been proven. In fact, as shown above, our patients are often mildly hypotonic.

The distribution of water within the body seems largely irrelevant since the water from glucose (or dextrose) containing solutions 'moves rapidly to equalise the osmotic concentration between cells and their surroundings' regardless (Michell *et al.* 1989).

Having said this, the preceding lines remain pure theorising. Undoubtedly, many clinicians have successfully used hypotonic fluids to treat sick reptiles although no objective data are available. Prezant & Jarchow (1997) recommend a 1:1 mixture of 5% dextrose and a non-lactated isotonic mammalian mixed electrolyte solution.

Lactic acid

Prezant & Jarchow (1997) also include a review of the literature relating to lactic acidosis in reptiles, including chelonians. They conclude that there is strong evidence that clinically-significant lactic acidosis is readily induced in reptiles, particularly by muscular activity such as struggling during blood sampling or stomach tubing, and is slow to resolve (hours to days) even at optimum body temperature. Lutz & Dunbar-Cooper (1987) showed that lactic-acid levels in loggerhead turtles (*Caretta caretta*) captured in trawl nets would not normalise for *at least* twenty hours. Lactic acidosis induces further electrolyte imbalances, impairs metabolic activity and reduces haemoglobin oxygen-carrying capacity. For this reason it was argued that it may be wise to avoid administering lactated fluids (e.g. Hartmann's solution) to chelonians. This view is controversial.

Potassium

In mammals, blood potassium levels above 7 mmol/l are thought to represent a threat to cardiac function (Michell *et al.* 1989). This reflects our experience that all animals with concentrations exceeding 6.9 mmol/l subsequently died. Administration of glucose-containing solutions induces the release of insulin that counters hyperkalaemia. Since potassium is predominantly an intracellular cation, hypokalaemic patients are likely to have a large deficit in total body potassium. Disturbances of this magnitude are probably best treated by the *oral* administration of potassium-containing preparations.

Selection of fluids for parenteral administration

Table 16.17 outlines the appropriate fluids to be used for parenteral administration to chelonians.

Table 16.17 Selection of fluids for parenteral administration.

Indication	Fluid of choice
Hypertonic dehydration (inability or lack of opportunity to drink)	0.18% NaCl/4% glucose (fast administration may risk cerebral oedema)
Isotonic or hypotonic dehydration (inability to drink *plus* chronic anorexia)	0.9% (normal) saline *or* 0.9% (normal) saline plus 5% glucose *or* 1:1 mixture of 5% dextrose:Ringer's solution
To achieve diuresis	0.18% NaCl/4% glucose
Blood loss	0.9% (normal) saline *or* whole blood
Septic shock	0.9% (normal) saline *or* Ringer's solution
Hyperkalaemia	5% glucose
Mixed water and electrolyte loss (diarrhoea)	Ringer's solution

Are marine turtles a special case?

Holmes and McBean (1964) found that the salt gland of marine turtles was capable of secreting a fluid of fixed sodium:potassium ratio, and that the kidneys represent a 'minor excretory source' for these cations. The elimination of these ions is therefore linked.

Since the potential diets of these species are high in potassium, these authors (RW & SM) hypothesised that it was necessary for them to drink sodium-rich sea water in order to facilitate potassium excretion. In fact, the mean plasma potassium concentration of turtles maintained in fresh water was 2.58 mEq/l as opposed to 1.48 mEq/l for those eating the same diet but swimming in salt water.

It might be expected that sick turtles receiving fluid therapy low in sodium could be at risk of hyperkalaemia—a potentially life-threatening imbalance. It would seem wise to monitor the plasma electrolyte levels of turtles requiring fluid therapy and to consider the possibility of administering relatively sodium-rich parenteral fluids, oral saline or even sea water itself orally (sodium content 460 mEq/l, potassium 7.6 mEq/l). Holmes & McBean calculated that even starved juvenile green turtles (*Chelonia mydas*) needed to drink 13 ml sea water/kg body weight to achieve electrolyte homeostasis.

In contrast to much of the above, Whitaker & Krum (1999) suggest that the options for intravenous/intracoelomic fluid therapy in marine turtles are:

- lactated Ringer's solution at 1%–3% of total body weight;
- lactated Ringer's solution/2.5% dextrose (1:1 ratio) at 1%–3% of total body weight;
- 2.5% dextrose/0.45% saline (2:1 ratio) at 1%–3% of total body weight,

and that 0.9% saline or fresh water, also at 1%–3% of total body weight, may be used for oral rehydration. No information is available on the success of such therapy in practice.

How much fluid should be given, how quickly and over what period?

Two considerations are important: replenishment of deficit and subsequently satisfaction of maintenance requirements.

There appears to be no scientifically-derived information on fluid requirements for sick reptiles. This has much to do with the problem of quantifying water deficits in reptiles (see above). A mixture of educated guesswork and experience has led to

Table 16.18 Evaporative water losses from two species of chelonian (Schmidt-Nielsen & Bentley 1966).

Species	Temperature	Pulmonary loss (ml/kg/day)	Transcutaneous loss (ml/cm^2/day)*
Gopherus agassizii	23°C	0.4	1.5
	35°C	1.6	2.1
Terrapene carolina	23°C	2.6	5.3

*where approximate surface area in cm^2 = 10 × weight in g$^{2/3}$

recommendations that fluids equivalent to 2%–5% of body weight should be administered to chelonian patients during initial stabilisation (Jarchow 1988; Page & Mautino 1990; Pokras *et al.* 1992; Krum 1997).

There are anecdotal reports that fluid equivalent to 5% of body weight may be administered intracoelomically to chelonians without compromising lung function.

The issue of maintenance fluid requirements for chelonians is not widely discussed in the literature. Many terrestrial species from arid environments are adapted to maximise uptake when water is available and then to survive long periods without further intake. Schmidt-Nielsen & Bentley (1966) demonstrated evaporative water loss (transcutaneous plus pulmonary) of 3 ml/day in a 1770 g desert tortoise (*Gopherus agassizii*) maintained at 25°C rising to almost 6 ml/day at 35°C. In a less arid-adapted species, the box turtle (*Terrapene carolina*), losses were 400% greater. Evaporative water loss is disproportionately greater in smaller individuals (Gans *et al.* 1968). An indication of the scale of potential evaporative losses for terrestrial chelonians can be gathered from Table 16.18.

Few authors have even ventured an opinion as to how rapidly parenteral fluids may be given. Page & Mautino (1990) recommend that the rate of administration should not exceed 1 ml/min but did not further expand upon this statement.

GASTROINTESTINAL MOTILITY MODIFIERS

Several authors, including Norton *et al.* (1989), have used metoclopramide at empirical doses in individual cases. Much the same is true for cisapride (Stein 1995: personal communication; Johnson *et al.* 1998). Tothill *et al.* (1999) investigated the effect of daily oral cisapride, metoclopramide or erythromycin on gut transit times in desert tortoises (*Gopherus agassizii*). No statistically-significant differences between the three treatments were recorded.

DIURETICS

Frye (1991a) suggests frusemide 5 mg/kg IM every 12–24 hours.

Diuresis may have a part to play in the management of hyperuricaemia, acute renal failure, and possibly even congestive cardiac failure. However, reptiles lack a loop of Henlé. Chelonians routinely produce hyposmotic urine anyway and have the capacity to resorb water from the bladder regardless of the composition of ureteral urine. The effect of frusemide in chelonians has yet to be demonstrated in practice.

HORMONES

Thyroid

Norton *et al.* (1989) described the successful management of a Galapagos tortoise (*Geochelone nigra*) that exhibited clinical signs and serum thyroid hormone levels compatible with either primary or secondary hypothyroidism. A good response was seen to improvement in nutrition, antibiosis and levothyroxine (Soloxine®, Daniels Pharmaceuticals) administration at 0.02 mg/kg PO every 48 hours. Cessation of thyroid supplementation led to relapse.

Glucocorticoids

Holt (1981) reviews some of the effects of the administration of corticosteroids to reptiles. However, at present we are unaware of any clinical indications for their use in chelonians.

Oxytocin

We have found a dose of 1 IU/kg to be safe and effective in the induction of oviposition. This dose may be repeated at intervals of 12 hours. A variety of doses and dose intervals have been reported (see Formulary).

Calcitonin

Frye (1991a) administered 1.5 IU/kg SC every 8 hours in the treatment of hypercalcaemia. However, Mader (1993) used the much higher dose of 50 IU/kg IM for each of two injections separated by one week to promote the incorporation of calcium into skeletal tissue in iguanas with metabolic bone disease. In these animals, it is essential to ensure that normocalcaemia is established, by prior vitamin D and calcium administration if necessary, before instigating calcitonin therapy, in order to avoid potentially fatal hypocalcaemia.

ANALGESICS

Anecdotal accounts of the use of non-steroidal anti-inflammatory drugs (NSAIDs) are available. Efficacy is very difficult to assess. Flunixin meglumine (Finadyne®, Schering) has been used at 0.1–0.5 mg/kg IM without obvious adverse effect (Mautino & Page 1993). In other species, more prostaglandin-sparing NSAIDs are now widely used. At Holly House surgery we have used carprofen (Rimadyl®, Pfizer) injectable at 2–4 mg/kg in a number of Mediterranean tortoises (McArthur 1999).

Opiates appear subjectively to be relatively ineffective in most reptiles. The reasons for this are poorly understood. Avery Bennet (1998) reports, however, that butorphanol (0.4 mg/kg IM) given 20 minutes before anaesthesia may decrease induction-agent requirements and provide sedation and analgesia. A combination of 0.4 mg/kg butorphanol with 2 mg/kg midazolam is also described. Heard (1993) suggests 0.2 mg/kg butorphanol to sedate *Gopherus agassizii*.

URATE METABOLISM AND EXCRETION

The use of drugs to modify urate metabolism and promote its excretion (uricosurics) remains speculative. Concepts and doses have simply been extrapolated from human gout therapy. No objective data are available on the results of therapy in tortoises.

Allopurinol is a xanthine oxidase inhibitor which reduces the conversion of hypoxanthine to xanthine and of xanthine to uric acid in the liver. *Probenecid* promotes active renal excretion of uric acid, however, the potential exists for damage to the renal tubules should uric acid precipitate in glomerular filtrate. *Sulphinpyrazone* is an alternative uricosuric. In man, these drugs are counter-productive in the treatment of acute gout but are useful in chronically-affected patients.

Patients in which we have used oral allopurinol have all been severely ill at the outset and have all ultimately died. This was also the experience of Martinez-Silvestre (1997) who treated a chronically inappetant, hyperuricaemic 1.15 kg *Testudo graeca* (which proved to have glomerulonephritis and visceral gout) with a combination of intracoelomic Ringer's solution, allopurinol 20 mg/kg PO every 24 hours and probenecid 250 mg PO every 12 hours. During three months of treatment this patient ate and was active. One month after cessation of therapy signs recurred and the animal died despite renewed treatment.

By contrast, Figueres (1997) successfully treated an inappetant 0.65 kg Mediterranean pond turtle (*Mauremys leprosa*) that was suffering articular gout with allopurinol 10 mg/kg PO every 24 hours for one month and then 3 mg/kg PO every 24 hours for six months in conjunction with a hypouricogenic diet (canine u/d®, Hills). In this case blood uric acid levels were not elevated and it may be that this animal had no renal pathology but had been fed an inappropriately high-protein diet. After five months of treatment, blood uric acid levels had fallen from 83 to 77 µmol/l and articular lesions were considered to have resolved.

VITAMINS

Vitamin A

A full discussion of hypo- and iatrogenic hypervitaminosis A is beyond the scope of this chapter. Suffice it to say that potentially fatal hypervitaminosis A may be induced relatively easily with injectable preparations—particularly aqueous solutions—and is common in patients arriving at our clinic. Some doses recommended in earlier literature have undoubtedly been excessive. This problem is compounded by the high concentration of vitamin A in commercially available preparations (which are designed for much larger species). Appropriate care should be taken in calculating dose rates. In many situations oral supplementation is entirely adequate.

Vitamin D

A full discussion of vitamin D metabolism, hyper- and hypovitaminosis D appears in the chapter on Nutrition. There appears to be no published data concerning vitamin D requirements of chelonians although there is widespread agreement that deficiency is common in captive animals. Supplementation remains somewhat more of an art than a science.

The term vitamin D covers a range of compounds that influence calcium and phosphorus metabolism. Ultraviolet B light is responsible for the photobiogenesis of vitamin D. Oral vitamin D supplements include vitamin D_2, vitamin D_3 and 25 hydroxycholecalciferol (25(OH)D). Care should be taken when using mixed vitamin A and D preparations not to induce hypervitaminosis A. Vitamin D toxicity is also a possibility. However, it should not be assumed that soft-tissue mineralisation is invariably a sign of hypervitaminosis D (Ullrey & Bernard 1999).

Doubts have been raised about the efficacy of oral vitamin D supplementation without appropriate lighting. Apparently healthy juvenile desert tortoises (*Gopherus agassizii*) and juvenile African spurred tortoises (*Geochelone sulcata*) housed indoors and fed diets containing about 2000 IU vitamin D_3/kg had serum concentrations of 25(OH)D less than 5 ng/ml (Bernard 1995). No measurable changes in serum levels were seen after oral dosing with 20,000 IU vitamin D_2/D_3 (desert tortoises) or 8.5 IU vitamin D_3/g body weight (*Geochelone sulcata*). This mirrors the situation in green iguanas where it has proved difficult to prevent metabolic bone disease with food supplements alone (Ullrey & Bernard 1999).

In contrast, Highfield (1996) found that juvenile tortoises could be maintained in a low ultraviolet environment without developing signs of metabolic bone disease provided that oral calcium and vitamin D supplementation were adequate. Dacke (1979) discussed the regulation of calcium in sub-mammalian vertebrates and concluded that solar irradiance is essential for *some* reptiles while dietary vitamin D is unimportant.

In summary, ultraviolet light *may* be unnecessary but at present it seems prudent to recommend that it be provided.

Vitamins D_2 and D_3 require hydroxylation in the kidney (at least in mammals) and may not be effective in animals with renal compromise. Of the hydroxylated vitamin D products, dihydrotachysterol (AT10®, Sanofi) has relatively rapid onset of activity in mammals (1–7 days) and duration of activity 1–3 weeks after discontinuation. Calcitriol and alfacalcidol are more rapid in onset (1–4 days) and have short half-life (<1 day). We have used dihydrotachysterol but not calcitriol or alfacalcidol.

MINERALS

Iodine

Large tortoises are susceptible to hypoiodinism if fed goitrogenic diets, in which case they require additional dietary sodium iodide in their diet several times weekly (Frye & Dutra 1974).

Sodium chloride

Marine turtles maintained in fresh water may benefit from additional dietary salt. See discussion in Fluid Therapy above.

NEBULISATION

As discussed in Respiratory Disease, chelonians cannot cough and lack a mucociliary escalator. Because of this, accumulation of exudate in the airways can seriously compromise ventilation. Hydration of this debris may help alleviate this problem.

Klingenberg (1996) describes the use of an avian nebuliser for respiratory infections in reptiles. He recommends two or three 30–45 minute treatment periods with normal saline each day for severely dyspnoeic animals. The potential benefit of the addition of the bronchodilator metaproterenol (2.5 ml of 0.6% in 3 ml saline) and/or the anti-secretagogue atropine (1 drop of 0.1 mg/ml solution in 3 ml saline) to the nebulised solution are discussed.

HYPERBARIC OXYGEN THERAPY (HBO)

To our knowledge, HBO has not been applied to chelonian medicine in practice. It seems worth mentioning, however, because chelonians frequently suffer from the kind of chronic disorders associated with immunocompromise or hypovascularity which in man have proved amenable to HBO. Such therapy is safe, non-invasive and, although requiring specialised facilities, should be viewed in the light of the potential cost of prolonged conventional therapeutic agents.

Mader (1991) discusses the application of HBO to human bacterial osteomyelitis—concluding that 60%–80% of patients who have failed to respond to surgical and/or antibiotic therapy will achieve remission. Similarly, Farmer (1991a) advocates HBO therapy of chronic human rhinocerebral mucormycosis, a fungal disease seen primarily in immunocompromised patients and associated with high mortality.

VACCINATION

Herpesvirus is probably responsible for many disease epidemics where animals from different sources are mixed. The risk of herpes-virus related disease outbreaks are a significant obstacle to breeding programmes and therefore herpesvirus is a prime candidate for vaccine development. The serological tools to detect carrier animals exist, but their reliability (sensitivity) remains to be established.

Recently, attempts to vaccinate against herpesvirus, using an inactivated, non-adjuvanted vaccine, have been made (Marschang *et al.* 2001). However, results at this early stage have not been successful.

FORMULARY

ROGER WILKINSON

Anaesthetic agents and sedatives are dealt with elsewhere in this volume.

The dose rates given below do not, in most cases, take account of individual body size. Formulae for the derivation of doses through allometric scaling have been described (Sedgwick & Borkowski 1996) although the validity of the use in chelonians of the energy constant appropriate for other reptilian orders remains to be demonstrated. Smaller individuals often require higher doses per unit of body weight. However, this is not invariably so. For example, Mautino & Page (1993) found that higher doses per unit of body weight of succinyl choline were required to achieve equivalent clinical effect in larger chelonians.

The inclusion of a therapeutic agent in this chapter does not necessarily signify that its use is advocated by the author.

The drugs are listed in alphabetical order.

ACYCLOVIR

Trade names: Zovirax®, Glaxo Wellcome.

Formulation: Oral: 200 or 400 mg/5ml suspension; topical: 5% cream.

Indications: Acyclovir is a pyrimidine analogue, which inhibits incorporation of thymidine into viral DNA. It has been used to treat tortoises suffering from clinical manifestations of herpesvirus infection.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Gopherus agassizii	80 mg/kg	24 hrs	PO	data not available	Klingenberg (1996)
Testudo spp	80 mg/kg	8 hrs	PO	data not available	McArthur (2002): personal communication

Contraindications and adverse effects: None known. We have used it in numerous animals at the above doses without apparent adverse effect.

Interactions: None known.

Comments: Nothing is known of the pharmacokinetics of acyclovir in reptiles. Nor have controlled studies of efficacy been undertaken although, subjectively, it seems to help some herpetic patients when used at eight-hour intervals. Results with once daily dosing have been disappointing in *Testudo horsfieldi* with upper respiratory tract disease from which herpesvirus was isolated (McArthur 2000). In man, capacity for absorption of acyclovir from the gut is readily saturated. Higher serum levels are achieved with more frequent dosing. To date, empirically derived oral doses have been used in chelonians. The topical 5% ointment has also been used in stomatitis cases (Cooper *et al.* 1988). In theory, this drug may be most effective when used early in the course of disease (during viral replication). Oral lysine therapy might also be considered in the management of herpesvirus disease (see Griffith *et al.* 1978).

ALLOPURINOL

Trade names: Allopurinol; Zyloric®, Glaxo Wellcome (United Kingdom).

Formulation: Oral: 100 mg and 300 mg tablets (Zyloric®).

Indications: Allopurinol is a xanthine oxidase inhibitor that blocks the formation of uric acid. In theory, it might be of benefit in the management of gout or hyperuricaemia in chelonians.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles[1], *Testudo graeca*[2]	20 mg/kg	24 hrs	PO	data not available	[1]Mader (1996b); [2]Martinez-Silvestre (1997)
Reptiles[1], *Mauremys leprosa*[2]	10 mg/kg	24 hrs	PO	data not available	[1]Mautino & Page (1993), 2Figueres (1997)
Chelonians	50 mg/kg	24 hrs	PO	data not available	Kölle (2001)

Contraindications and adverse effects: In man this drug is contraindicated in acute gout, renal dysfunction or hepatic dysfunction—which would account for a large proportion of our patients! Pancreatitis and reversible hepatopathy are potential adverse-effects in man.

Special precautions: Ensure adequate hydration. In fact, induction of diuresis may be desirable where renal function allows.

Comments: No pharmacokinetic data are available for chelonians. Figueres (1997) achieved clinical cure in an inappetant 0.65 kg European pond turtle (*Mauremys leprosa*) with articular gout using allopurinol at a dose derived from that used in the dog, by allometric scaling, and by feeding a hypouricogenic diet (canine u/d®, Hills). This animal was not hyperuricaemic at the time of presentation and blood uric acid levels did not fall significantly during treatment. Its gout may have resulted from excess dietary protein rather than from renal pathology. The same success has not yet been reported for hyperuricaemic patients with renal pathology.

AMIKACIN

Trade names: Amikacin; Amikin®, Bristol-Myers (United Kingdom).

Formulation: Injectable: 500 mg/2ml vial.

Indications: An aminoglycoside antibiotic: particularly useful in the treatment of Gram-negative infections.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Testudo hermanni	50 mg/kg	12 hrs	IM	data not available	Spörle *et al.* 1991

Contraindications and adverse effects: Potentially nephrotoxic and ototoxic.

Special precautions: Use with care in the presence of renal dysfunction. Ensure adequate hydration.

Interactions: β-lactam antibiotics may inactivate aminoglycosides *in vitro*. They may, however, be synergistic *in vivo*. Avoid concurrent use of other potentially nephrotoxic drugs such as frusemide (furosemide), amphotericin, or probenecid.

Comments: Inactive in low-oxygen sites and in the presence of organic debris. Anaerobes are thus uniformly resistant—a problem which can be overcome by the concurrent use of metronidazole or a β-lactam. Adequate hydration is important to avoid nephrotoxicity but overhydration may lead to accelerated elimination and loss of therapeutic effect. Fluid balance must be critically assessed. Synergy with both third generation cephalosporins and semi-synthetic penicillins has been demonstrated (see under ceftazidime).

AMPICILLIN

Trade names: Penbritin Injectable®, GlaxoSmithKline (United Kingdom).

Formulation: Injectable: 500 mg vial for reconstitution.

Indications: A β-lactam antibiotic. Most Enterobacteriaceae are resistant except *Salmonella arizona* and some *Proteus mirabilis*. Ampicillin is a good choice where anaerobes are involved and also often effective against *Dermatophilus*.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Testudo hermanni	50 mg/kg	12 hrs	IM	data not available	Spörle *et al.* 1991

Contraindications and adverse effects: None significant.

Interactions: Do not mix with aminoglycosides *in vitro* or inject at the same site.

Comments: Bactericidal but β-lactamase susceptible. Renally excreted—elimination may be slower in dehydrated animals or Species from marine or arid terrestrial environments than in freshwater aquatic Species.

BUTORPHANOL

Trade names: Torbugesic®, Fort Dodge (United Kingdom).

Formulation: Injectable: 10 mg/ml.

Indications: A partial agonist opioid with sedative and analgesic effects.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Gopherus agassizii	0.2 mg/kg	Once	IM	data not available	Heard (1993)
Chelonians	0.4 mg/kg	Once	IM	data not available	Bennett (1998b)

Contraindications and adverse effects: Reduced doses may be appropriate in animals with impaired renal or hepatic function.

Interactions: May potentiate the effects of other sedatives or anaesthetics.

Comments: Bennett (1998) reports that butorphanol given 20 minutes before anaesthesia may decrease induction-agent requirements and provide sedation and analgesia. A combination of 0.4 mg/kg butorphanol with 2 mg/kg midazolam is also described. Opioid antagonists such as naloxone might be used to reverse the effects of butorphanol.

CALCITONIN (SALCATONIN®)

Trade names: Calsynar®, R.P.R. (United Kingdom).

Formulation: Injectable: 100 or 200 IU/ml.

Indications: Salcatonin is synthetic salmon calcitonin. It is responsible for net movement of calcium from the blood into bone and may therefore be of benefit in the management of metabolic bone disease. However, lizards may be more in need of aggressive therapy for this condition than chelonians since they are more susceptible to skeletal problems. In man, salmon calcitonin is less immunogenic than that of porcine origin. Fifty units of salmon calcitonin are equivalent to 80 IU of porcine calcitonin.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Iguana iguana	50 IU/Kg	Day 0 and 7	IM	data not available	Mader (1993)
Chelonians	1.5 IU/Kg	?once	IM	data not available	Highfield (1996)

Contraindications and adverse effects: Ensure normocalcaemia as calcitonin treatment will result in potentially fatal depression of blood calcium.

Special precautions: A short course of calcium and vitamin D_3 should precede calcitonin treatment.

Interactions: None known.

CALCIUM GLUCONATE/BOROGLUCONATE

Trade names: Calcium gluconate or borogluconate.

Formulation: Injectable: 100 mg/ml solution.

Indications: Hypocalcaemia or induction of ovulation.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	10 mg/kg borogluconate	24 hrs for up to 5 days before oxytocin	IM	data not available	Highfield (1996)
Chelonians	100 mg/kg gluconate	24 hrs	IM	data not available	Mautino & Page (1993)

Contraindications and adverse effects: May cause tissue reaction after IM or SC injection.

Interactions: Do not add to electrolyte solutions containing bicarbonate salts.

Comments: We administer calcium at least a few hours before induction of ovulation with oxytocin.

CARBENICILLIN

Trade names: Pyopen®, GlaxoSmithKline (United Kingdom).

Formulation: Injectable: 1 g vials for reconstitution.

Indications: A semi-synthetic penicillin antibiotic with enhanced anti-Gram-negative activity.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Testudo graeca & T. hermanni	400 mg/kg	48 hrs	IM	data not available	Lawrence *et al.* (1986)

Contraindications and adverse effects: None significant.

Interactions: Inactivated on *in vitro* contact with aminoglycosides.

Comments: Biphasic serum level increase after single injection (Lawrence *et al.* 1986). Vulnerable to the rapid development of resistance—for this reason best used in combination with other antibiotics. Other semi-synthetic penicillins, such as piperacillin or ticarcillin, may have improved anti-pseudomonal effect.

CARPROFEN

Trade names: Rimadyl®, Pfizer (United Kingdom).

Formulation: Injectable: 50 mg/ml solution.

Indications: A prostaglandin-sparing, non-steroidal anti-inflammatory used primarily as an analgesic; particularly useful peri-operatively.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Testudo spp.	4 mg/kg	Once	SC	data not available	McArthur unpublished observations

Special precautions: Particular care is necessary when treating animals with hepatic or renal disease or with reduced renal perfusion.

Interactions: avoid concurrent use of other potentially nephrotoxic drugs such as aminoglycosides or amphotericin.

Comments: In the United States this drug is available only as a tablet. Doses used to date have simply been extrapolated from mammalian data. No information is available on efficacy or pharmacokinetics in reptiles.

CEFOPERAZONE

Trade names: Cefobid or Magnamycin, Pfizer (USA).

Formulation: 250 mg, 1 g, 2 g vials for reconstitution.

Indications: This is a third-generation cephalosporin antibiotic that is more lipophilic than ceftazidime and thus more widely-distributed in the body. It is eliminated principally by hepatic metabolism rather than renally as for ceftazidime. It may also be even less β-lactamase susceptible than ceftazidime.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Gopherus polyphemus	5 mg/kg	48 hrs	IM: site not specified	30°C	Caligiuri *et al.* (1990)

Contraindications and adverse effects: None of significance.

Interactions: May be synergistic *in vivo* with aminoglycosides and fluoroquinolones.

Comments: Particularly useful in patients with possible renal compromise for which aminoglycosides would otherwise be a good choice. A particular advantage over fluoroquinolones and aminoglycosides is efficacy against anaerobes. No side effects have been observed to date. Once reconstituted into solution it can be frozen in aliquots of the required dose and thawed for injection. Mayer & Nagy (1999) investigated the synergistic effects of aminoglycosides (amikacin and netilmicin) in combination with third-generation cephalosporins (cefoperazone, ceftriaxone and ceftazidime) against 18 clinical human isolates of *Pseudomonas* spp. Using a disc-diffusion method it was demonstrated that amikacin or netilmicin in combination with one of the three cephalosporins exhibited synergy against 7–12/18 isolates. When a quinolone (ciprofloxacin, ofloxacin or pefloxacin) was used in combination with a third-generation cephalosporin (cefoperazone, ceftriaxone or ceftazidime) synergistic effects were seen against 3–5/18 isolates. No antagonism was found with these combinations.

CEFTAZIDIME

Trade names: Fortum®, GlaxoSmithKline (United Kingdom).

Formulation: Injectable: 250 mg and 500 mg vials for reconstitution.

Indications: Ceftazidime is a third-generation cephalosporin antibiotic with enhanced anti-Gram-negative activity, including *Pseudomonas* spp.; a β-lactam with resistance to β-lactamase.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Testudo hermanni	50 mg/kg	12 hrs	IM	data not available	Spörle *et al.* 1991

Contraindications and adverse effects: None of significance.

Interactions: May be synergistic *in vivo* with aminoglycosides and fluoroquinolones.

Comments: Ceftazidime is particularly useful in patients with possible renal compromise for which aminoglycosides would otherwise be a good choice. A particular advantage over fluoroquinolones and aminoglycosides is efficacy against anaerobes. No side effects have been observed to date. Once reconstituted into solution it can be frozen in aliquots of the required dose and thawed for injection. Mayer & Nagy (1999) investigated the synergistic effects of aminoglycosides (amikacin and netilmicin) in combination with third-generation cephalosporins (cefoperazone, ceftriaxone and ceftazidime) against 18 clinical human isolates of *Pseudomonas* spp. Using a disc-diffusion method it was demonstrated that amikacin or netilmicin in combination with one of the three cephalosporins exhibited synergy against 7–12/18 isolates. When a quinolone (ciprofloxacin, ofloxacin or pefloxacin) was used in combination with a third-generation cephalosporin (cefoperazone, ceftriaxone or ceftazidime) synergistic effects were seen against 3–5/18 isolates. No antagonism was found with these combinations.

CHLORAMPHENICOL

Trade names: Chloramphenicol: Kemicetine®, Pharmacia & Upjohn (United Kingdom).

Formulation: Injectable: 1 g vials for reconstitution (Kemicetine®).

Indications: Chloramphenicol is a bacteriostatic antibiotic with a broad spectrum of activity including against Gram-positives, Gram-negatives, anaerobes, *Chlamydia* spp. and *Mycoplasma* spp. Enterobacteriaceae often acquire resistance. It is widely distributed within the body.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Gopherus agassizii	0.2 mg/kg	Once	IM	data not available	Heard (1993)
Chelonians	0.4 mg/kg	Once	IM	data not available	Bennett (1998b)

Contraindications and adverse effects: Potential for bone marrow suppression. This effect, well documented in mammals, has not been demonstrated in reptiles to date although a very depressed PCV was seen in one chloramphenicol-treated snake (Clark *et al.* 1985).

Special precautions: Dose adjustments may be necessary in patients with renal or hepatic compromise.

Interactions: None of significance.

Comments: Handle with care to minimise human exposure.

CHLOROQUINE

Trade names: Avlochlor®, Zeneca; Nivaquine, R.P.R. (United Kingdom).

Formulation: Oral: 150 mg tablets and 50 mg/5ml syrup; injectable: 40 mg/ml.

Indications: Chloroquine is an anti-protozoal that exerts its effect through preventing parasite nucleic acid synthesis. It may also have immunomodulating properties. Most widely used as an anti-malarial in human medicine. Although tortoises may not suffer from plasmodial infections, they are subject to other protozoal haemoparasites such as *Haemoproteus* spp. Chloroquine is also effective against amoebic trophozoites (though not cysts) and extra-intestinal amoebiasis.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	50 mg/kg	weekly	IM	data not available	Innis (personal communication)

Special precautions: Care in impaired renal or hepatic function.

Interactions: None of significance.

Comments: The only amoebicide currently available in injectable form. Current knowledge suggests that *Haemoproteus* infections are usually non-pathogenic but limited information is available.

CISAPRIDE

Trade names: Prepulsid®, Janssen (United Kingdom).

Formulation: Oral: 10 mg tablets or 1 mg/ml suspension.

Indications: A prokinetic agent used in the management of gastrointestinal stasis.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Not specified	1 mg/kg	24 hrs	oral	no data available	Johnson *et al.* (1998)
Gopherus agassizii	1 mg/kg	24 hrs	oral	no data available	Tothill *et al.* (1999)

Contraindications and adverse effects: Gastrointestinal discomfort has been reported in mammals. The incidence of serious ventricular arrhythmias in human patients has led to the withdrawal of cisapride from the market in many parts of the world.

Interactions: Cardiac effects have been reported in man when combined with drugs altering its elimination (e.g. clarithromycin) or in susceptible individuals.

Comments: The above dosages are empirical. No pharmacokinetic data is available for reptiles and efficacy has not been demonstrated (Tothill *et al.* 1999).

CLARITHROMYCIN

Trade names: Klaricid®, Abbott (United Kingdom).

Formulation: Oral: 500 mg sustained-release tablets; 250 mg and 500 mg tablets; 250 mg powder sachets; 125 mg/5ml suspension; injectable: 500 mg vial for reconstitution and injection.

Indications: Clarithromycin is a macrolide antibiotic with bacteriostatic or bactericidal activity depending on concentration and microbial susceptibility. Its spectrum of activity is similar to that of penicillin—including Gram-positive cocci, Gram-positive bacilli, some Gram-negative bacilli, such as *Pasteurella*, some *Chlamydia* spp., some mycoplasmata and non-tubercular *Mycobacteria* (when used in combination with fluoroquinolones).

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Gopherus agassizii	15 mg/kg	48–72 hrs	PO	not given	Wimsatt *et al.* (1999)

Contraindications and adverse effects: In man, adverse effects have included stomatitis, glossitis and cholestatic hepatitis.

Special precautions: Use with care in animals with hepatic impairment.

Interactions: In vivo synergy against *Mycobacteria* is seen when fluoroquinolones are used concurrently.

Comments: A subject of current interest due its potential application in the treatment of upper respiratory tract disease in *Gopherus agassizii*.

CLINDAMYCIN

Trade names: Cleocin phosphate®, Pharmacia and Upjohn (United States); Dalacin C®, Pharmacia and Upjohn (United Kingdom).

Formulation: Injectable: 150 mg/ml ampoules for injection (Dalacin C®).

Indications: Clindamycin is a lincosamide antibiotic whose spectrum includes many anaerobes and Gram-positive cocci.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Caretta caretta	5 mg/kg	24 hrs	IM	no data available	Nutter *et al.* (2000)

Contraindications and adverse effects: Rapid IV injection can lead to cardiac depression and neuromuscular blockade in other Species. Potentially fatal gastrointestinal disturbances may arise in man, rabbits and rodents.

Interactions: May potentiate the effects of non-depolarising muscle-relaxants and oppose the action of neostigmine.

Comments: Also available in various oral formulations. Nothing is known of the pharmacokinetics of this drug in chelonians. In the Reference cited, the authors used this drug for 14 days to treat a *Clostridium difficile* infection which had been demonstrated on culture. The patient recovered.

DIMETRIDAZOLE

Trade names: Emtryl Soluble for Game Birds®, Merial (United Kingdom).

Formulation: Oral: 40% w/w powder.

Indications: An anti-protozoal.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	40 mg/kg	24 hrs	PO	not given	Lawrence (1985)

Contraindications and adverse effects: Over-dosage in other Species results in undesirable neurological signs; a suspected carcinogen in man; avoid inhalation of powder.

Interactions: None of significance.

Comments: Not available in the United States. Holt (1981) suggested five days' treatment for flagellates, seven days' for hexamitiasis and eight days' for amoebiasis.

DIOCTYL SULPHOSUCCINATE (DOCUSATE SODIUM)

Trade names: Dioctyl®, Schwarz; Docusol®, Typharm (United Kingdom).

Formulation: Oral: 50 mg/5ml or 12.5 mg/5ml solution.

Indications: A laxative used in the treatment of gastrointestinal obstruction.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Giant tortoises	0.3 ml/kg	24 hrs for 7 days	PO	no data available	Norton *et al.* (1989)

Contraindications: None of significance.

Interactions: None of significance.

DOXYCYCLINE

Trade names: Ronaxan®, Merial; Vibramycin®, Pfizer; Doxycycline (United Kingdom).

Formulation: Oral: 20 mg, 100 mg tablets/capsules.

Indications: Doxycycline is a tetracycline antibiotic with activity against bacteria, mycoplasmata, *Chlamydia* spp., *Mycobacteria* and some protozoa. In human medicine, doxycycline is used in malaria prophylaxis. It is widely distributed within the body and is excreted in faeces.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Testudo hermanni	50 mg/kg	initial dose	IM	no data available	Spörle *et al.* 1991
	25 mg/kg	subsequently	IM	no data available	

Contraindications and adverse effects: Rarely of significance.

Interactions: Absorption from the gut is reduced by calcium, magnesium and iron salts.

Comments: Absorption after oral administration may be unpredictable. Doxycycline may be subject to more rapid elimination when administered orally or by injection in the caudal half of the body due to hepatic pre-systemic elimination.

EDTA (SODIUM CALCIUM EDETATE)

Trade names: Ledclair®, Sinclair (United Kingdom).

Formulation: Injectable: 5 ml vial of 200 mg/ml solution.

Indications: Lead poisoning.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelydra serpentina	2 mg/kg	every 10 days	IM	no data available	Borkowski (1997)

Special precautions: Renal function should be monitored since both lead and EDTA are potentially nephrotoxic.

Comments: Borkowski's patient (10 kg body weight) had ingested a lead fishing sinker and was initially very depressed. The sinker was surgically removed, blood lead levels fell from 3.5 ppm to the limit of detection during 40 days of EDTA treatment and the turtle recovered and was subsequently released to the wild. Enrofloxacin and fluids were also administered. The EDTA dose was derived by allometric scaling from recommended mammalian and avian treatment regimes.

ENROFLOXACIN

Trade names: Baytril®, Bayer (United Kingdom).

Formulation: Oral: 25 mg/ml or 100 mg/ml solution; injectable: 25 mg/ml or 50 mg/ml.

Indications: Enrofloxacin is a veterinary fluoroquinolone licensed for use in exotic Species in the United Kingdom. It is bactericidal and broad spectrum with efficacy against Gram-negative and Gram-positive bacteria plus *Mycobacteria*. It has poor activity against anaerobes. Enrofloxacin is widely distributed within the body.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Testudo hermanni	10 mg/kg	24 hrs	IM	no data available	Spörle *et al.* (1991)
Terrapene carolina	5 mg/kg	every 4–5 days	IM	no data available	Aucoin, quoted in Jacobson (1999a)
Geochelone elegans	10 mg/kg	24 hrs for most bacteria	IM	no data available	Raphael *et al.* (1994b)
Geochelone elegans	10 mg/kg	12 hrs for *Pseudomonas* and *Citrobacter*	IM	no data available	Raphael *et al.* (1994b)
Gopherus polyphemus	5 mg/kg	24–48 hrs	IM	no data available	Prezant *et al.* (1994)

Contraindications and adverse effects: Cartilage abnormalities may occur when fluoroquinolones are administered to growing mammals. It is unknown whether this might be relevant when long courses are administered to young chelonians.

Interactions: Probenecid blocks the renal elimination of fluoroquinolones and may lead to elevated blood levels.

Comments: Enrofloxacin also achieves comparable blood levels after oral administration (Vancutsem *et al.* 1990). Synergy with cephalosporins may occur (see under ceftazidime).

FENBENDAZOLE

Trade names: Panacur®, Hoechst (United Kingdom).

Formulation: Oral: 25 mg/ml or 100 mg/ml suspension; 187 mg/g paste.

Indications: Fenbendazole is an anthelminthic effective against a wide range of nematode infestations, except perhaps *Proatractis* spp. It is ovicidal and also has some efficacy against certain cestodes.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	30–50 mg/kg for nematodes; 50–100 mg/kg for spirurids	Once or more; Holt (1981) recommends day 0 and 21	PO	no data available	Frank & Reichel (1977)
Chelonians	100 mg/kg	Once	Intracolonic	no data available	Innis (1995)

Contraindications and adverse effects: None of significance: 'Overdosage almost impossible' (Frank & Reichel 1977). Concerns have recently been raised by reports of increasing susceptibility to adverse reactions in bird collections.

Interactions: None of significance

Comments: Efficacy of single oral dose against oxyurids is unpredictable. Better results may be achieved with daily doses of 10–30 mg/kg over two weeks (Frank & Reichel 1977) or by dosing intracolonically (Innis 1995). Highfield (1996) suggests that individuals which are difficult to stomach tube are given paste formulation on a selected item of food. Intracolonic dosing is an alternative answer to this problem.

FLUCONAZOLE

Trade names: Diflucan®, Pfizer (United Kingdom)

Formulation: Oral: 50 mg or 200 mg capsules; 50 mg or 200 mg/5 ml suspension; injectable: 2 mg/ml solution for infusion.

Indications: A triazole antifungal with broad-spectrum activity although variably effective against *Aspergillus* and *Penicillium*. It is widely distributed in the body including the CNS, saliva and respiratory tract. Unlike ketoconazole, it is not metabolised in the liver but is excreted via the urine achieving high concentrations

here. In man it is particularly used in the treatment of candidiasis at all mucosal sites and fungal infections of the urinary tract.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Sea turtles	data not available	data not available	data not available	data not available	Whitaker & Krum (1999)

Special precautions: Use with care in the presence of renal compromise.

Interactions: None of significance in reptiles to date.

Comments: Used with 'safety and efficacy' in combination with enrofloxacin 'over several months' (Whitaker & Krum 1999).

FLUNIXIN MEGLUMINE

Trade names: Finadyne®, Schering-Plough (United Kingdom).

Formulation: Injectable: 10 mg/ml.

Indications: A potent non-steroidal anti-inflammatory which is less prostaglandin-sparing than its successors such as carprofen. It has been used in mammalian medicine particularly in the treatment of endotoxaemia.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	0.1–0.5 mg/kg	data not available	data not available	data not available	Mautino & Page (1993)

Contraindications and adverse effects: In mammals, nephrotoxicity, hepatotoxicity and gastrointestinal ulceration have been associated with the use of flunixin. It should not be used in hypotensive patients, in combination with other potentially nephrotoxic drugs or in animals with pre-existing renal disease.

Interactions: Do not use in combination with other potentially nephrotoxic drugs such as aminoglycosides or amphotericin.

Comments: Not a drug that we have used in the treatment of chelonians. A prostaglandin-sparing NSAID would seem a better choice in most circumstances.

FRUSEMIDE (UNITED KINGDOM), FUROSEMIDE (UNITED STATES)

Trade names: Dimazon®, Intervet (United Kingdom).

Formulation: Injectable: 50 mg/ml.

Indications: A diuretic that in mammals acts upon the loop of Henlé. Diuresis may have a part to play in the management of hyperuricaemia, acute renal failure, and possibly even congestive cardiac failure in chelonians. However, reptiles lack a loop of Henlé. Chelonians routinely produce hyposmotic urine anyway and have the capacity to resorb water from the bladder regardless of the composition of ureteral urine.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	5 mg/kg	12–24 hrs	IM	data not available	Frye (1991a)

Contraindications and adverse effects: Potential impact upon renal function should be taken into account. It would appear unwise to use this drug in combination with other potentially nephrotoxic agents.

Interactions: May reduce the elimination of other drugs normally excreted through the kidneys.

Comments: The effect of frusemide in chelonians has yet to be demonstrated in practice.

GENTAMICIN

Trade names: Genticin®, Roche; Cidomycin®, Hoechst (United Kingdom).

Formulation: Injectable: 40 mg/ml solution.

Indications: An aminoglycoside antibiotic with bactericidal effect. Particularly effective against Gram-negative bacteria, although some Gram-positives are also sensitive; ineffective against anaerobes and thus often used in combination with a β-lactam or metronidazole.

Species	Dose	Frequency	Route	Temperature	Reference
Chrysemys picta	10 mg/kg	48 hrs	IM	26°C	Bush *et al.* (1977)
Chrysemys scripta elegans	6 mg/kg	single dose lasts 2–5 days	IM	data not available	Raphael *et al.* (1985)

Contraindications and adverse effects: Potentially nephrotoxic and ototoxic; contraindicated in renal insufficiency. If renal function is questionable then measurement of blood levels may be useful if there is no alternative antibiotic. In mammals it is recommended that the trough serum level should not fall below 1 mg/ml. Aminoglycosides as a group have pronounced post-antibiotic effect and pulse dosing is an acceptable option.

Interactions: Do not use in combination with other potentially nephrotoxic drugs such as NSAIDs or amphotericin.

Comments: Inject in the front half of body if possible.

IODOQUINOL (DIIODOHYDROXYQUIN)

Trade names: Yodoxine®, Glenwood Palisades, Tenafly, NJ (United States).

Formulation: May be available as powder.

Indications: An amoebicide effective against enteric trophozoites and cysts; may be used alone, or in combination with metronidazole.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	50 mg/kg	24 hrs for 21 days	PO	data not available	Innis (personal communication)

Contraindications and adverse effects: None reported in chelonians. In man, neurological signs are reported. In juvenile black rat snakes, Denver *et al.* (1999) reported splenitis, pancreatitis and hepatitis when iodoquinol was administered at 30–120 mg/kg/day for 14 days.

Interactions: None of significance

ITRACONAZOLE

Trade names: Sporanox®, Janssen (United Kingdom).

Formulation: Oral: 10 mg/ml solution; 100 mg capsules; injectable: an injectable solution is available in the United Kingdom for human treatment on a named patient basis.

Indications: A triazole antifungal agent with activity against *Candida, Aspergillus, Coccidioides* and *Cryptococcus.* Unlike fluconazole, it does not achieve high concentrations in saliva or cerebrospinal fluid (CSF).

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chamaeleo spp.	10 mg/kg	24 hrs for 21 days	PO	data not available	Paré *et al.* (1997)
Spiny lizard	23.5 mg/kg	24 hrs for 3 days	PO in food	data not available	Gamble *et al.* (1997)

Contraindications and adverse effects: Potentially hepatotoxic in mammals. In birds it appears to have a narrow safety margin and may cause anorexia. One of the chameleons treated by Paré *et al.* became inappetant towards the end of treatment, and was shown to have hepatomegaly. Fungal skin lesions were successfully resolved at 10 mg/kg daily. It died one year later and cholecystitis and septicaemia were demonstrated. The other animal died six days into therapy but was also systemically affected by mycosis. No hepatic changes were apparent in this case.

Interactions: None of apparent significance.

Comments: Experience in chelonians with this drug is limited to a handful of individual cases.

KETOCONAZOLE

Trade names: Nizoral®, Janssen (United Kingdom).

Formulation: Oral: 200 mg tablets; 100 mg/5ml oral suspension.

Indications: An imidazole antifungal with efficacy against *Candida, Aspergillus* and Coccidioides.

Species	Dose	Frequency	Route	Temperature	Reference
Gopherus polyphemus	15–30 mg/kg	24 hrs	PO	data not available	Page *et al.* (1991)

Contraindications and adverse effects: Eliminated largely by hepatic metabolism but also potentially hepatotoxic.

Special precautions: Use with care in the presence of possible hepatic insufficiency.

Interactions: None of apparent significance.

Comments: The oral suspension is convenient for administration to chronically-unwell animals with a semi-permanent oesophagostomy tube. For example, secondary yeast infections may contribute to morbidity in patients with oral ulcerations resulting from herpesvirus. We have treated numerous such tortoises in this way for periods of up to two months without apparent adverse effect.

LEVAMISOLE

Trade names: Levacide®, Norbrook laboratories; Levadin®, Vétoquinol; Nilverm®, Schering-Plough (United Kingdom).

Formulation: Oral 30 mg/ml or 15 mg/ml solution; injectable: 75 mg/ml injection.

Indications: An anthelminthic with neurotoxic effects on nematodes.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Tortoises	50 mg/kg	once	PO	data not available	Zwart & Ham (1972)
Tortoises	300 mg/kg	once	PO	data not available	Frank (1976)

Contraindications and adverse effects: A variety of potential adverse effects is described in mammals. Gastrointestinal signs are most often encountered.

Interactions: Toxicity may be potentiated by organophosphate exposure.

Comments: Unsatisfactory efficacy against tortoise ascarids and oxyurids (Moser 1973).

LEVOTHYROXINE

Trade names: Soloxine®, Daniels (United Kingdom).

Formulation: Oral: 0.05, 0.1, 0.2, 0.3, 0.5, 0.8 mg tablets.

Indications: A synthetic thyroxin used in the treatment of primary or secondary hypothyroidism. There are anecdotal reports of its use in patients with apparent hepatic lipidosis.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Giant tortoises	0.02 mg/kg	24 hrs	PO	data not available	Norton *et al.* (1989)

Contraindications and adverse effects: None of apparent significance.

Interactions: None of apparent significance.

Comments: This dose appears to be extrapolated from the commonly used starting dose in canine hypothyroidism. Norton *et al.* describe the treatment of one tortoise with primary or secondary hypothyroidism.

LYSINE

Indications: An amino acid which, in human medicine, has been used successfully to reduce the incidence of relapse in herpes simplex.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Man	300–1200mg	24 hrs	PO	data not available	Griffiths *et al.* (1978)

Contraindications and adverse effects: None of apparent significance.

Interactions: None of apparent significance.

Comments: Dietary arginine antagonises therapeutic effect. To the author's knowledge, lysine has not yet been applied to the treatment of chelonian herpesvirus. However, it would appear to have potential.

MEBENDAZOLE

Trade names: Telmin®, Janssen (United Kingdom).

Formulation: Oral: 100 mg/g granules; 100 mg tablets.

Indications: A benzimidazole anthelminthic effective against nematodes and many cestodes.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	25 mg/kg	once	PO	data not available	Soifer (1978)

Contraindications and adverse effects: None of apparent significance.

Interactions: None of apparent significance.

Comments: Holt *et al.* (1979) considered it ineffective in a tortoise at 50 mg/kg.

MEDROXYPROGESTERONE ACETATE

Trade names: Depo-Provera®, Pharmacia & Upjohn (United Kingdom).

Formulation: Injectable: 150 mg/ml.

Indications: A progestagen that has been used to suppress ovarian activity. This is of potential benefit in the management and possibly the prevention of follicular stasis—a potentially fatal, chronic disease of female chelonians that appear to lack the cues for ovulation (McArthur 2000).

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	'mammalian dose'	Twice a year	SC	data not available	Frye (1991a)

Contraindications and adverse effects: Potentially diabetogenic. In practice, appears to be well tolerated.

Interactions: None of apparent significance.

Comments: Frye (1991a) stated that the above dose would completely suppress ovarian activity. The author has used proligestone as an alternative (see below).

METOCLOPRAMIDE

Trade names: Emequell®, Pfizer; Maxolon®, Monmouth (United Kingdom).

Formulation: Oral: 10 mg tablets; 1 mg/ml syrup; injectable: 5 mg/ml injection.

Indications: An anti-emetic and gastrointestinal motility modifier. In mammals it inhibits vomiting by acting at the chemoreceptor trigger zone and increases oesophageal sphincter tone. Peristaltic activity in the stomach, duodenum, and jejunum is increased. These effects have not been demonstrated in chelonians (or other reptiles).

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Giant tortoises	0.06 mg/kg	24 hrs	PO	data not available	Norton *et al.* (1989)
Gopherus agassizii	1 mg/kg	24 hrs	PO	data not available	Tothill *et al.* (1999)

Contraindications and adverse effects: Do not use in cases of gastrointestinal obstruction. Has the potential to cause sedation, disorientation and extra-pyramidal motor disorders. In mammals renal blood flow may be reduced.

Interactions: CNS effects may be exacerbated by other sedatives or anaesthetics. Opiates may oppose its gastrointestinal effects.

Comments: Tothill *et al.* (1999) found that gut passage time was unaffected even at 1 mg/kg. It should not necessarily be assumed, however, that gut passage time is a good indicator of all components of gut behaviour. Doses have been empirically derived.

METRONIDAZOLE

Trade names: Flagyl®, R.P.R. (United Kingdom).

Formulation: Oral: 200 mg/5ml suspension; injectable: 500 mg/100ml injection.

Indications: A synthetic nitro-imidazole that is widely distributed within the body, has good penetration of abscesses and activity against both bacteria and protozoa. It is particularly effective against anaerobes. Although effective against amoebic trophozoites and extra-intestinal amoebiasis, enteric cysts may not be eliminated by metronidazole alone. Absorption after oral dosing is good. Metabolised by the liver prior to renal excretion

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles (antibacterial)	50 mg/kg	24 hrs	PO	data not available	Klingenberg (1996)
Reptiles (antiprotozoan)	260 mg/kg	Once	PO	data not available	Bone (1992)
Reptiles (antiprotozoan)	160 mg/kg	24 hrs for 3 days	PO	data not available	Jackson (1976)
reptiles (antiprotozoan)	100 mg/kg	days 1 and 14	PO	data not available	Jacobson (1999)
green iguana and yellow rat snake	20 mg/kg	48 hrs	PO	data not available	Kolmstetter *et al.* (1997 & 1998)

Contraindications and adverse effects: CNS disturbances and hepatotoxicity are reported in mammals. Pre-existing hepatic dysfunction may increase the risk of nervous system problems.

Interactions: None of apparent significance.

Comments: The studies of Kolmstetter *et al.* represent the only pharmacokinetic studies available. Stewart (1990) reported that all of his anaerobe isolates from reptiles were sensitive to metronidazole (*Bacteroides, Fusobacterium, Clostridium and Peptostreptococcus*). Klingenberg (1993) notes that prophylactic treatment of Asian box turtles (*Cuora* sp.) with metronidazole at transportation 'dramatically improves survival rates'. Metronidazole may be combined with iodoquinol in an attempt to eradicate amoebal infection.

MILBEMYCIN

Trade names: Interceptor®, Novartis (United States).

Formulation: Oral and Injectable: Bodri *et al.* (1993) used powdered Interceptor tablets suspended in propylene glycol to give a 2.5 mg/ml solution. This appeared to be suitable for oral or subcutaneous administration.

Indications: A macrocyclic lactone parasiticide effective against many nematodes and arthropods but not cestodes or trematodes. It binds glutamate-gated chloride channels and GABA receptors resulting in paralysis of sensitive organisms.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Box turtles and freshwater turtles	0.5–1 mg/kg	Days 1 and 8	PO (or SC but see comments below)	data not available	Bodri *et al.* (1993)

Contraindications and adverse effects: No adverse effects noted in 19 animals (*Pseudemys scripta* and *Terrapene carolina*) by Bodri *et al.* (1993).

Interactions: Insufficient information.

Comments: The injectable preparation used by Bodri *et al.* is not commercially available. Only very preliminary data on efficacy has been presented. Acanthocephalans appear to be non-susceptible (Bodri *et al.* 1993).

NATAMYCIN

Trade names: Mycophyt® (Intervet) is a topical preparation licensed for use in cattle and horses (United Kingdom).

Formulation: Not currently available as a systemic treatment in the United Kingdom.

Indications: Enteric candidiasis.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	3 mg/kg	24 hrs	PO	data not available	Zwart & Buitelaar (1980)

Contraindications and adverse effects: None of apparent significance.

Interactions: None of apparent significance.

Comments: Not systemically absorbed.

NEOMYCIN

Trade names: Neobiotic®, Pharmacia & Upjohn (United Kingdom).

Formulation: Oral: 700 mg/g neomycin sulphate soluble powder.

Indications: An aminoglycoside antibiotic. As a class, aminoglycosides are bactericidal, effective against many Gram-negative bacteria and some Gram-positives but are ineffective against anaerobes. Neomycin is very nephrotoxic and is used primarily as a topical or oral preparation. It is not absorbed from the gut. Thorsen (1974) used neomycin in tank water to eliminate Salmonellae, with apparent success, from a group of freshwater chelonians.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	65 mg/kg	twice	PO	data not available	Highfield (1996)

Contraindications and adverse effects: Use with extreme care in animals with renal compromise as nephrotoxicity has been reported in mammals even with oral treatment where gastrointestinal ulceration exists. Potential for nephrotoxicity may be greater in terrestrial or salt-water chelonians.

Special precautions: Do not use in conjunction with other potentially nephrotoxic drugs.

Comments: Alternative aminoglycosides (gentamicin, amikacin) are preferred in most (if not all) situations for parenteral treatment.

NYSTATIN

Trade names: Nystan®, Squibb (United Kingdom).

Formulation: Oral: 100,000 IU/ml suspension.

Indications: A broad-spectrum antifungal used principally for the treatment of oral or gastrointestinal candidiasis. Not absorbed after oral administration.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	100,000 IU/kg	24 hrs	PO	data not available	Jacobson (1980)

Contraindications and adverse effects: None of significance.

Interactions: None of significance.

Comments: Possibly the drug of choice for the treatment of gastrointestinal candidiasis in very sick patients with hepatic and renal compromise.

OXFENDAZOLE

Trade names: Systamex®, Schering-Plough (United Kingdom).

Formulation: 22.65 mg/ml or 90.6 mg/ml suspension.

Indications: A benzimidazole anthelminthic effective against many nematodes and some cestodes; ovicidal.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Terrapins	200–400 mg per 5 gallons of tank water			29.5°C	Thorsen (1974)

Contraindications and adverse effects: None of apparent significance.

Interactions: None of apparent significance.

Comments: Various authors have suggested that oxfendazole has a wider safety margin than fenbendazole (e.g. Highfield 1996). The basis of this assertion is unknown to this author. In mammals, fenbendazole is metabolised to oxfendazole and both are active drugs. The capacity for interconversion in chelonians is unknown.

OXYTETRACYCLINE

Trade names: Duphacycline®, Fort Dodge; Embacycline®, Merial; Oxycare®, Animalcare (United Kingdom).

Formulation: Injection: 50 mg/ml or 100 mg/ml solution.

Indications: A bacteriostatic antibiotic with activity against many Gram-positive and Gram-negative bacteria, mycoplasmata and spirochaetes.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Gopherus agassizii	5–10 mg/kg	24 hrs	IM	data not available	Gauvin (1993)
Tortoises	50 mg/animal	24 hrs for 6 days	PO	data not available	Weber (1973)

Contraindications and adverse effects: In birds, muscle necrosis has occurred at injection sites. In mammals, a variety of adverse effects are sometimes seen, including gastrointestinal disturbances, hepatotoxicity and, most importantly, a dose-dependent renal tubular nephrosis which may be exacerbated by dehydration or concurrent administration of other potentially-nephrotoxic drugs.

Interactions: Avoid concurrent administration of other potentially nephrotoxic drugs. The action of bactericidal antibiotics may be reduced by concurrent tetracycline administration.

Comments: Has been used in mycoplasmosis cases 'with some success' (Johnson *et al.* 1998). No pharmacokinetic data is available for chelonians. Weber's dose was apparently effective in eliminating latent *Salmonella* infection. Oxytetracycline is less lipid-soluble than doxycycline and is less widely distributed in the body. It is excreted unchanged in urine and bile. Absorption after oral administration is unpredictable.

OXYTOCIN

Trade names: Oxytocin-S®, Intervet (United Kingdom).

Formulation: Injectable: 10 IU/ml solution.

Indications: Stimulates smooth muscle contraction and promotes oviposition.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	1 IU, 1.5–10 IU and <3 IU have all been advised	once or more	IM	data not available	Cooper *et al.* (1991)
Chelonians	1–10 IU/kg	once	IM or IC (intracoelomic)	data not available	DeNardo (1996)
Chelonians	2–10 IU/kg	repeat in 4–8 hrs if necessary	IM	data not available	Highfield (1996)

Contraindications and adverse effects: Do not use if obstruction to passage of eggs has not been corrected. Prior radiography is always advisable. Care should be taken as oviductal spasm and rupture can occur at higher dosages.

Interactions: None of significance.

Comments: The presence of calcified eggs in a tortoise is not necessarily an indication for intervention. Where delayed oviposition is thought to be a threat to the patient's well-being, it is vital to consider environmental factors. In many cases it is these, rather than muscular inertia, which have led to egg retention. Where recourse to oxytocin therapy is necessary, it may be wise to administer calcium initially. Highfield recommends a five-day course of calcium injections. At what interval repeat injections of oxytocin should be administered is a matter for speculation. We have had good results with one daily dose for up to three days. Care should be taken to avoid the eggs being crushed by the mother if she is in a confined space.

Arginine vasotocin is the reptilian hormone analogous to mammalian oxytocin. It appears to be more effective than oxytocin in provoking smooth muscle contraction and will stimulate oviposition in some dystocia cases that have previously failed to respond to oxytocin (Lloyd 1990). See Chapter 13 for a more detailed discussion of dystocia management including the use of beta-blockers.

PAROMOMYCIN

Trade names: Humatin®, Parke-Davis (United States).

Indications: An aminoglycoside antibiotic that is not absorbed from the gut under normal circumstances; potential in the elimination of *Cryptosporidia* and in the amoebic cysts but would not be expected to have efficacy against extra-intestinal amoebiasis.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Gila monster (cryptosporidiosis)	300–360 mg	48 hrs for 14 days	PO	data not available	Pare *et al.* (1997)
Snakes (amoebiasis)	25–100 mg	24 hrs for 4 weeks	PO	data not available	Lane & Mader (1996)

Contraindications and adverse effects: Potentially nephrotoxic.

Specific precautions: Use with care in the presence of questionable gut wall integrity or renal dysfunction.

Interactions: None of known significance.

Comments: Experience with this drug in chelonians is lacking.

POTASSIUM (CHLORIDE/BICARBONATE)

Trade names: Various, including Sando-K®, Sandoz (United Kingdom).

Formulation: Oral: effervescent tablets providing K^+ 12 mmol, Cl^- 8 mmol.

Indications: Correction of hypokalaemia; oral dosing is the route of choice.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Testudo graeca	5 mmol/kg	once, then as dictated by blood potassium response	PO	25–30°C	Wilkinson, unpublished

Contraindications and adverse effects: Hyperkalaemia!

Interactions: None of significance.

Comments: Blood levels are the tip of the whole-body-potassium iceberg. Potassium is principally an intracellular cation. Hypokalaemic animals are likely to have a sizeable total deficit that cannot safely be replaced by parenteral therapy. In acidotic animals, intracellular potassium is displaced into the blood. Normokalaemia may mask a significant whole body deficit.

PRAZIQUANTEL

Trade names: Droncit®, Bayer (United Kingdom).

Formulation: Oral: 50 mg tablets; injectable: 56.8 mg/ml solution.

Indications: A cestocide with some effect against trematodes.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	5–30 mg/kg	once	PO or IM	data not available	Frank & Reichel (1977)
Chelonians	5–8 mg/kg	once	PO	data not available	Highfield (1996)
Loggerhead sea turtle (*Caretta caretta*)	25 mg/kg	3 times with three hours between doses	PO	data not available	Jacobson *et al.* (2002)

Contraindications and adverse effects: Well tolerated, no side effects noted up to 100 mg/kg (Frank & Reichel 1977).

Interactions: None of significance.

Comments: Clinical efficacy against spirorchids (digenetic trematodes) in green turtles (*Chelonia mydas*) is reported by Adnyana *et al.* (1997). The conclusion of the study by Jacobson *et al.* (2002) represents the only dose rate grounded in anything other than educated guesswork.

PROBENECID

Trade names: Benemid®, MSD (United Kingdom).

Formulation: Oral: 500 mg tablets.

Indications: A uricosuric that promotes renal excretion of uric acid. Used in the management of gout and hyperuricaemia.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles[1], *Testudo graeca*[2]	250 mg/animal	every 12 hrs	PO	data not available	[1]Mader (1996), [2]Martinez-Silvestre (1997)

Contraindications and adverse effects: In human medicine its use is contraindicated during episodes of acute gout.

Special precautions: Adequate hydration *must* be ensured to avoid precipitation of uric acid in the renal tubules.

Interactions: Do not use with other potentially nephrotoxic drugs such as frusemide or aminoglycosides.

Comments: 'Increase as needed' (Mader 1996). No pharmacokinetic data exists for chelonians. Mader's dose appears to be extrapolated from human medicine. In the only published case study on the use of probenecid, Martinez-Silvestre (1997) found that the patient ate and was active during three months of treatment, but ultimately succumbed to renal pathology one month after cessation of combined allopurinol/probenecid treatment. Since the animal was hyperuricaemic at the outset it seems reasonable to assume that the renal pathology probably pre-dated medical intervention.

PROLIGESTONE

Trade names: Delvosteron®, Intervet (United Kingdom).

Formulation: Injectable: 100 mg/ml suspension.

Indications: A synthetic progestagen that has been used to suppress ovarian activity. This is of potential benefit in the management and possibly the prevention of follicular stasis—a potentially fatal, chronic disease of female chelonians which appear to lack the cues for ovulation (McArthur 2000).

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Mediterranean *Testudo*	20 mg/kg	once	SC	data not available	McArthur (2000)

Contraindications and adverse effects: Potentially diabetogenic. In practice, appears to be well tolerated.

Interactions: None of apparent significance.

Comments: In our experience this dose is variably effective in the treatment of established cases of follicular stasis. Ultrasonographic monitoring has revealed subsequent regression of follicles in some cases. However, complete clinical cure has not been achieved to date with medical therapy alone and surgical ovariosalpingectomy remains the most reliable treatment.

SULPHADIMETHOXINE

Trade names: Coxi Plus®, Vetrepharm (United Kingdom).

Formulation: Oral: 1000 mg/4g powder in sachets licensed for administration to pigeons in the United Kingdom.

Indications: Although this sulpha drug has some antibacterial effect, combinations with trimethoprim are more effective. Sulphadimethoxine is used as a coccidiostat.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	50 mg/kg	24 hrs for 5–7 days then 48 hrs as needed	PO	data not available	Klingenberg (1995)

Special precautions: Use with care in animals with hepatic or renal compromise. Ensure adequate hydration to avoid crystal precipitation in the upper urinary tract.

Interactions: Unknown.

Comments: Considered by Klingenberg (1996a) to be the treatment of choice for coccidiosis in chelonians.

TRIMETHOPRIM/SULPHADIAZINE

Trade names: Delvoprim®, Intervet; Duphatrim®, Fort Dodge; Tribrissen®, Schering-Plough (United Kingdom).

Formulation: Injectable: 40 mg trimethoprim and 200 mg sulphadiazine/ml.

Indications: Synergistic antibacterial action effective against Gram-negative bacilli, some Gram-positive bacteria, and many coccidia. In chelonians this drug has been used most often in the treatment of coccidiosis. Many important chelonian bacterial pathogens (Enterobacteriaceae) are resistant. Trimethoprim/sulpha drugs are ineffective in the presence of necrotic tissue.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	30 mg/kg then 15 mg/kg	24 hrs, 2 days then 48 hrs × 5	IM	data not available	Lane & Mader (1996)
Testudo hermanni	30 mg/kg (combined total)	24 hrs for first two doses then 48 hrs	not given	data not available	Spörle *et al.* (1991)

Contraindications and adverse effects: A variety of predictable adverse reactions such as keratoconjunctivitis sicca and thyroid depression are seen in mammals but have not, to date, been reported in reptiles.

Special precautions: Use with care in animals with hepatic or renal compromise. Ensure adequate hydration to avoid crystal precipitation in the upper urinary tract.

Interactions: None of apparent significance.

Comments: Jacobson (1999a), Page & Mautino (1990) give similar recommendations except that the first two doses are also given 48 hours apart.

TYLOSIN

Trade names: Tylacare®, Animalcare; Tylan®, Elanco (United Kingdom).

Formulation: Oral: 200 mg tablets; injectable: 50 or 200 mg/ml.

Indications: A macrolide antibiotic with bacteriostatic effect on Gram-positive bacteria. It is particularly effective against mycoplasmata.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	25 mg/kg	24 hrs	IM or PO	data not available	Murphy (1973)
Reptiles	5 mg/kg	24 hrs	IM	data not available	Jacobson (1999a)

Contraindications and adverse effects: Gastrointestinal disturbances have occurred in mammals.

Interactions: None of apparent significance.

VITAMIN A

Iatrogenic hypervitaminosis A is a potentially fatal condition, readily induced with injectable preparations—particularly aqueous solutions. Appropriate care should be taken in calculating dose

rates. In many situations, oral supplementation is entirely adequate, particularly in terrestrial chelonians.

Trade names, preparations	Vitamin A content
Abidec drops, Warner Lambert	4,000 IU/0.6ml
Vionate, Vetark	220,000 IU/kg
Dandelion leaves	14,000 IU/100g (Highfield 1996)
Vitamin A Palmitate, Cambridge Labs (United Kingdom)	50,000 IU/ml
Aquasol A injection (United States)	50,000 IU/m

Indications: Hypovitaminosis A.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Chelonians	1,500–2,000 IU/Kg	Weekly × 2–6	SC	data not available	Boyer (1996)

Contraindications and adverse effects: Parenteral injection of doses of vitamin A carries a significant risk of causing hypervitaminosis A.

Interactions: None of significance but see comment on nephrotoxic drugs below.

Comments: Hypovitaminosis A is undoubtedly common in freshwater aquatic chelonians kept in captivity. Its incidence in terrestrial tortoises is less clear. Quite possibly many of these are receiving sub-optimal levels of vitamin A but the signs, which are often chronic and subtle, may respond well to oral supplementation. Unfortunately, the most definitive diagnosis available antemortem relies upon liver biopsy and a presumptive diagnosis is often made. Concurrent use of nephrotoxic drugs should probably be avoided since renal tubular pathology may also result from lack of vitamin A.

Hypervitaminosis A is characterised by epidermal sloughing leading to potentially fatal secondary infection and loss of fluids. It is difficult to induce this syndrome with oral vitamin A since gut absorption and hepatic metabolism provide a natural control. Palmer *et al.* (1984) found that *Testudo hermanni* injected with a single dose of an aqueous vitamin A preparation at 100,000 IU/kg developed physical signs of hypervitaminosis A, whilst those given a single dose of 100,000 IU/kg vitamin A in an oily vehicle did not. Where multiple doses are given, the threshold for toxicity becomes less predictable. The dose recommended by Boyer (1996) relates to an aqueous solution (Aquasol A). The vitamin A palmitate preparation available in the United Kingdom is formulated in castor oil. A wide range of dose rates are available in the literature, however many of these do not take account of body weight and often do not give details of the preparation used.

VITAMIN B_1 (THIAMINE)

Trade names: Various multivitamin preparations.

Indications: Thiamine deficiency in aquatic, carnivorous chelonians associated with a fish diet containing thiaminase.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	25 mg/kg	24 hrs	PO or SC	data not available	Frye (1991a)

Contraindications and adverse effects: None known.

Interactions: None known.

Comments: The above dose is somewhat empirical but appears to be effective. Response to treatment has been said to be 'generally dramatic' (Bennett 1996). Dietary deficiencies should be rectified.

VITAMIN D

Trade names:

	Vitamin A (IU)	Vitamin D_3 (IU)	Calcium (mg)	Phosphorous (mg)	Ca:P Ratio
Vionate® (Novartis)	220	22	94.5	63.6	1.46:1
SA 37® (Intervet)	769	76.9	10	10	1:1
Nutrobal® (Vetark UK)	500	150	208	4.5	46:1
ACE High® (Vetark UK)	2530	20	9.9	4.9	2:1
Arkvits® (Vetark UK)	1177	118	142	4.65	30:1

Indications: Prevention and treatment of metabolic bone disease (MBD). The following, empirically derived doses, have been used in the treatment of MBD.

Dose:

Species	Dose	Frequency	Route	Temperature	Reference
Reptiles	100 IU/kg	Days 0 and 7	IM	data not available	Donoghue & Langenberg (1996)
Chelonians	1–4 IU/kg D_3	24 hrs	PO	data not available	Highfield (1996)

Contraindications and adverse effects: In theory, calcification of soft tissues might occur with over-supplementation. In practice this appears to be uncommon.

Interactions: None known.

Comments: There are no reliable data for maintenance vitamin D requirements in chelonians—partly because this will vary with

ultraviolet light provision and dietary vitamin D, calcium and protein content. Overdosage appears to be uncommon with oral supplementation. Exposure to natural-spectrum ultraviolet light is important in maintaining vitamin D levels. However, Highfield (1996) reports that juvenile tortoises maintained in low-ultraviolet conditions showed no clinical manifestations of hypovitaminosis D when receiving oral calcium and vitamin D_3 supplementation. Unsupplemented tortoises were susceptible to hypovitaminosis D when ultraviolet light performance proved unreliable. This is in contrast to the situation in iguanas, which seem to have an absolute requirement for ultraviolet light to avoid metabolic bone disease.

APPENDICES

APPENDIX A: TURTLE CONSERVATION

(Stuart McArthur)

THREATS TO TURTLE POPULATIONS

Threats to turtle populations include: predation, infectious diseases, trauma and entanglement, ingestion of sea debris and, most importantly, habitat destruction (Fig. 18.1).

In South East Asia and many other areas turtle eggs are regarded as a potent aphrodisiac and rich nutrient source (Figs 18.2–18.3). Historically, turtles have been prized for their meat. The most famous use of which is turtle soup (Figs 18.4–4a).

Beaches reserved for turtle conservation are often plagued with sea debris (Fig. 18.5). Without legal protection through local planning controls, hotel complexes, airports, road and town lights derange hatchling routes to the sea. Light and noise pollution force gravid females to reject beaches for oviposition, often jettisoning their eggs in the sea or laying below the high-tide waterline. Where beaches are no longer considered viable, relocation of eggs as described later becomes an important conservation technique.

Recreational use of parasols and sun beds (which may spear and cool incubating nests), 4 × 4 traffic on beaches (which crushes nests) and mock-ecotourism (which fails to respect the needs of laying females and hatchlings) threatens to degrade all but the most remote of turtle beaches (Fig. 18.6).

Fig 18.1 The Turtle Survival Alliance and the Marine Turtle Specialist group are both working to help conservation groups trying to preserve terrestrial/semi-aquatic and marine chelonians respectively. Readers are encouraged to support such groups whenever possible.

Fig. 18.2 These turtle eggs were on sale 15 m from the main turtle station at Terengganu, Malaysia. Throughout my visit to the area, ten bags of ten eggs each were regularly on sale each morning. All were gone by the evening.

Fig. 18.3 The sale of turtle products is unfashionable and generally illegal. However they still appear on the walls of many restaurants throughout the world.

Fig. 18.4 Turtles are still farmed for their meat in the Caribbean.

Fig. 18.6 Many beaches historically frequented by egg-laying female turtles are now unusable as a result of hotel developments and beach-holiday tourism.

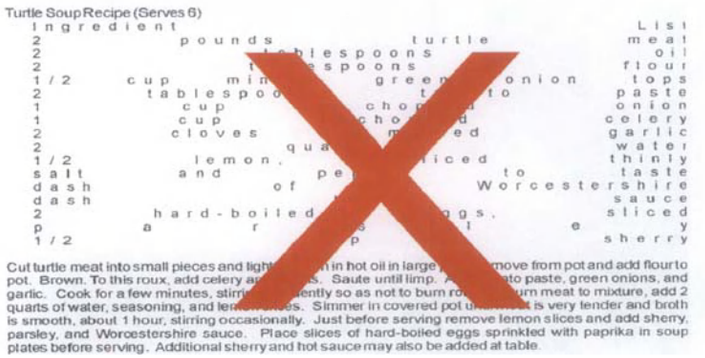


Fig. 18.4a

Fig. 18.7 Sightings of free-swimming turtles are now less commonplace.

Fig. 18.5 Beaches reserved for turtle conservation are often plagued with sea debris (Terengganu, Malaysia). This will result in entanglement of laying females when they come ashore.

Fig. 18.8 Turtle Conservation Centre, Terengganu, Malaysia. This is a site of global importance with respect to conservation of leatherback turtles (*Dermochelys coriacea*) and green turtles (*Chelonia mydas*).

Turtle conservation projects

The future of many turtle populations lies in the hands of individuals involved in conservation projects. Many of these projects now cooperate globally with other habitat protection, disease research and public education projects, regarding conservation of turtles (Fig. 18.7).

Government and charitable funding of these projects and the effective control and policing of planning regulations in turtle-laying areas are crucial to the survival of local populations (Fig. 18.8).

Turtles are now at greatest risk in less-developed countries with low per-capita incomes, where turtle populations are still significant. Here conservation education at local level is crucial to turtle survival. Beach protection signs must be clear, and official in nature. Leaflets can be distributed to local inhabitants and holidaymakers providing clear guidelines regarding the use of turtle-nesting beaches in protected areas (Figs 18.9–18.11).

Fig. 18.9 Terengganu, Malaysia (as in Fig. 18.7).

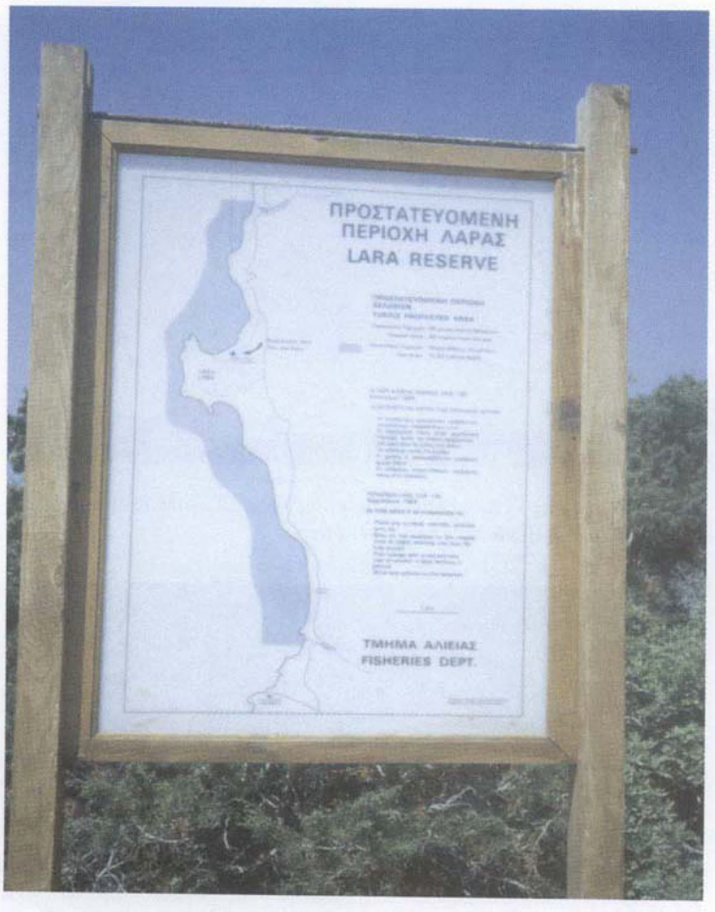


Fig. 18.10 Akamas National Park, Cyprus.
This is the location of long term intensive conservation management of green turtles (*Chelonia mydas*) in the Mediterranean.

Fig. 18.11 Kephalonia, Greece.
This beach is typical of beach conservation in areas of heavy commercial development. In the Mediterranean, where tourism has a high priority, loggerhead turtles (*Caretta caretta*) do not seem to be as valued as green turtles (*Chelonia mydas*).

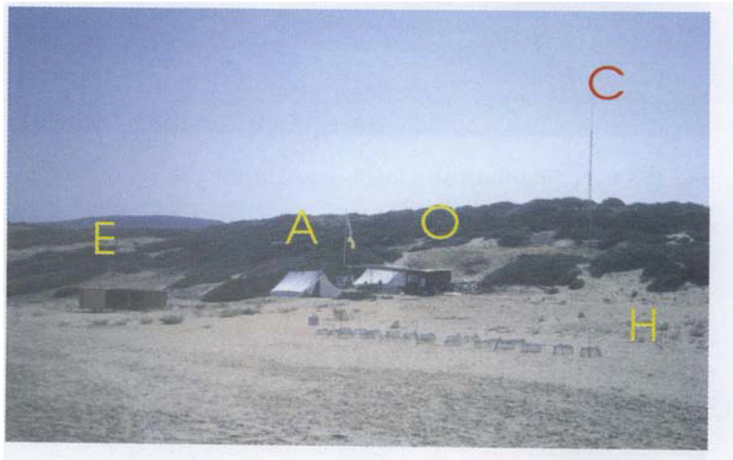


Fig. 18.12 The turtle station at Lara Beach in the Akamas National Park, Cyprus.
Most turtle stations provide workers with basic requirements. The accommodation here (A) is in tents and outdoor cooking, washing and toileting is necessary. There is a public education area (E), a radio communications tower (C), a sheltered beach-observation area (O) where the beach and hatchery (H) are easily monitored.

EGG RELOCATION

Nests are identified. Following careful location of the egg chamber, eggs are collected. In Cyprus this is often within 12 hours of their being laid. Transfer into a sand-lined thermos flask at this early stage means that maintaining egg orientation may not yet be crucial, however it is easily done. Reversal of the egg depth position in the flask allows correction when eggs are emptied out into a chamber in the hatchery. This may be important with respect to sex determination and incubation temperature/conditions within the egg chamber. Flasks can be maintained at suitable temperatures and transported in the evenings and mornings, at times when temperature extremes and vehicle vibrations can be minimised (Figs 18.12–18.15).

Nests in unsuitable areas can be located by beach patrols with night binoculars, appropriate following and marking of false and true nest crawls (turtle tracks), and information provided by the local population. Reasons for nest relocation include excessive predation from crabs, foxes and dogs, excessive beach development and other inappropriate locations (e.g. too near to the high tide mark or known 4 × 4 routes).

Fig. 18.13 A turtle nest is located and the egg chamber identified.

Fig. 18.14 A cylindrical flask is ideally suited to transportation of turtle eggs. The base is first packed with sand.

Fig. 18.15 The eggs are handled carefully in order to avoid unnecessary rotation, vibration and other trauma.

Eggs can be relocated to safe hatcheries where they are protected and monitored during their incubation period. They are replaced into egg chambers created to mimic those of the female turtle (Figs 18.16–18.18).

Hatcheries also give the facilities to head-start turtle hatchlings. Head-starting remains controversial, as husbandry requirements of wild chelonians are expensive and complex. Inappropriate

Fig. 18.16 The turtle hatchery at Lara beach, Cyprus, the site of egg relocation.

Fig. 18.17 The new chamber is easiest dug by hand using an action similar to that of the female turtle's flippers.

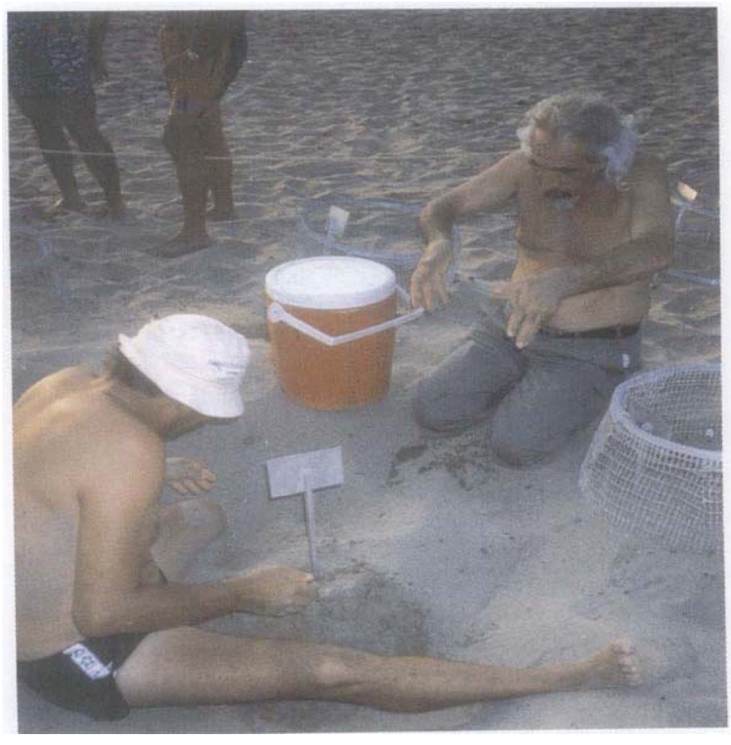


Fig. 18.18 The turtle egg chamber is recreated.

Fig. 18.19 This nest is examined 12 hours after emerging hatchlings are no longer observed.

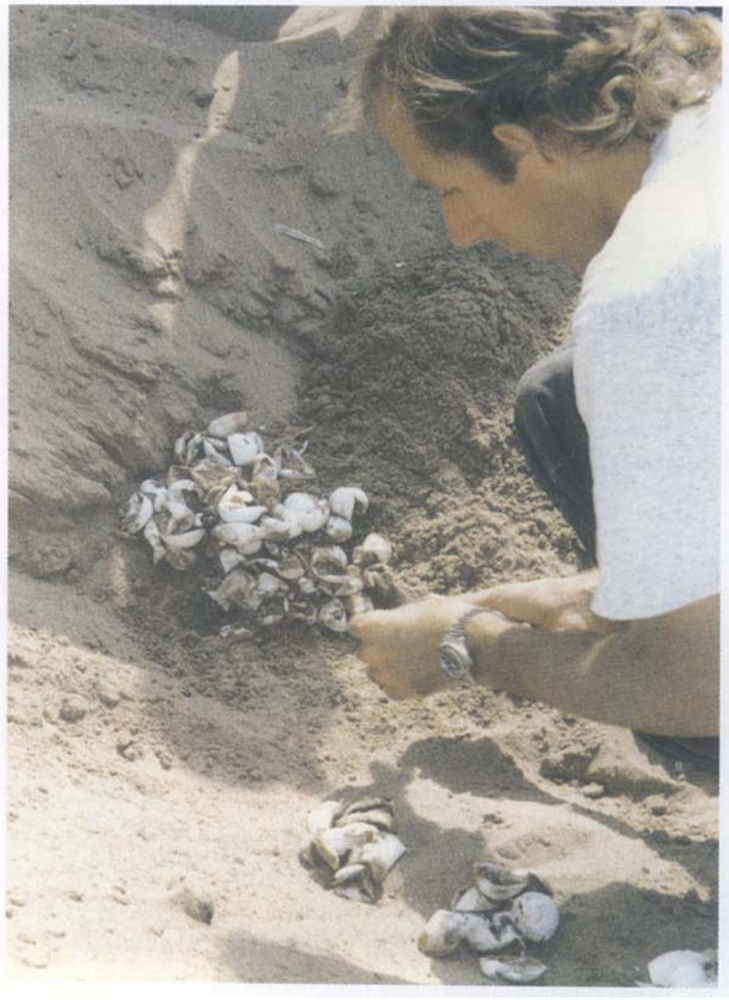


Fig. 18.20 Hatching rates, other nest statistics and future return emergence rates of mature females at the release beach give evidence of success.

head-starting may expose hatchlings to nutritional and infectious diseases and it is hard to maintain suitable water quality and temperature.

It is unclear whether beach imprinting during first descent from nest to the sea is crucial in the later return of gravid females of many species to the very nesting beach where they were themselves hatched. If this descent is important, it may be inappropriate to house turtles in pens for long periods. Where it is considered beneficial, the turtles should be allowed to descend and enter the water before being recaptured and moved to a head-start facility (Figs 18.19–18.21).

TURTLE TREATMENT AND REHABILITATION FACILITIES

Education, surgical and diagnostic facilities are housed here. Nursing and hospitalisation facilities are shown later. A dedicated ambulance allows rapid response to turtles in distress and return of rehabilitated turtles to suitable locations. Transportation of turtles has been described earlier. Treatment facilities should allow adequate exposure to natural sunlight but simultaneous protection from excessive exposure. Netting is therefore appropriate (Figs 18.22–18.24).

TURTLE NESTING

Figures 18.25–18.29 show a nesting green turtle (*Chelonia mydas*).

Fig. 18.21 A lucky hatchling is found trapped and alone, deep near the bottom of the egg chamber.

This hatchling was allowed to descend to the sea in the evening, after a short period of recovery, and out of the heat of the midday sun.

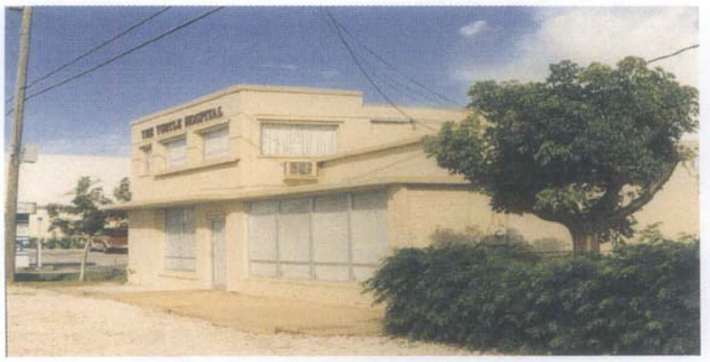


Fig. 18.22 The Turtle Hospital at Marathon, Florida.

Fig. 18.23 A dedicated turtle ambulance.

Fig. 18.24 The turtle nursing facility at Marathon Turtle Hospital, Florida.

Fig. 18.25 A nesting green turtle (*Chelonia mydas*).

Fig. 18.26 A female may excavate a large furrow before choosing the location of the egg chamber.

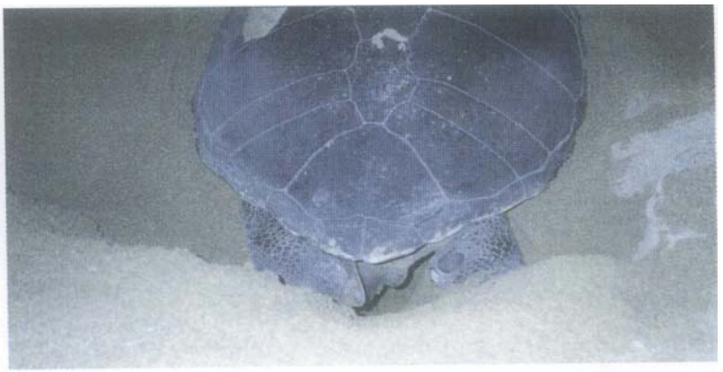


Fig. 18.27 The egg chamber is created by the digging action of the hind flippers.

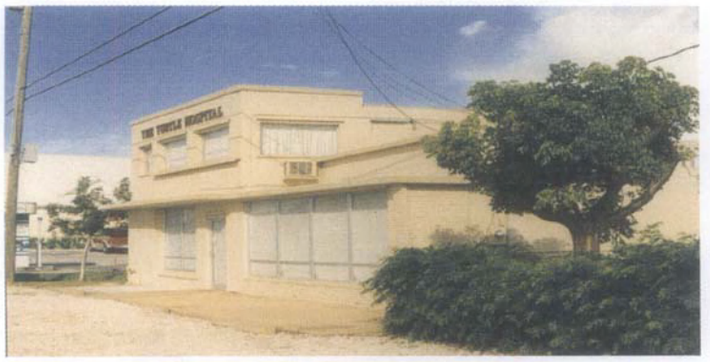
(Stuart McArthur)

Table 18.1 is an *anecdotal list* derived from several sources:

(1) Tortoise Trust Feeding Guide;
(2) British Chelonian Group: Feeding guide: *Testudo* **3** (1);
(3) Frye (1991b): Table 3.8 (Toxic plants) from *Tortuga Gazette* January 1982.

(Stuart McArthur and Michelle Barrows)

Below are examples of care plans for specific species or families. It is beyond the scope of this text to provide care plans for all species likely to be encountered. The examples cover an omnivorous, semi-aquatic, basking and hibernating species and a terrestrial



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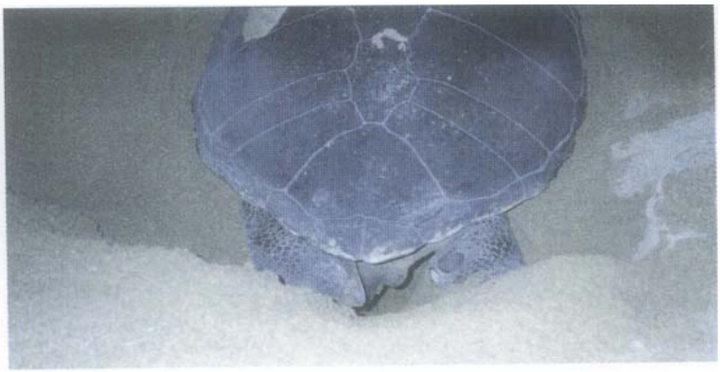

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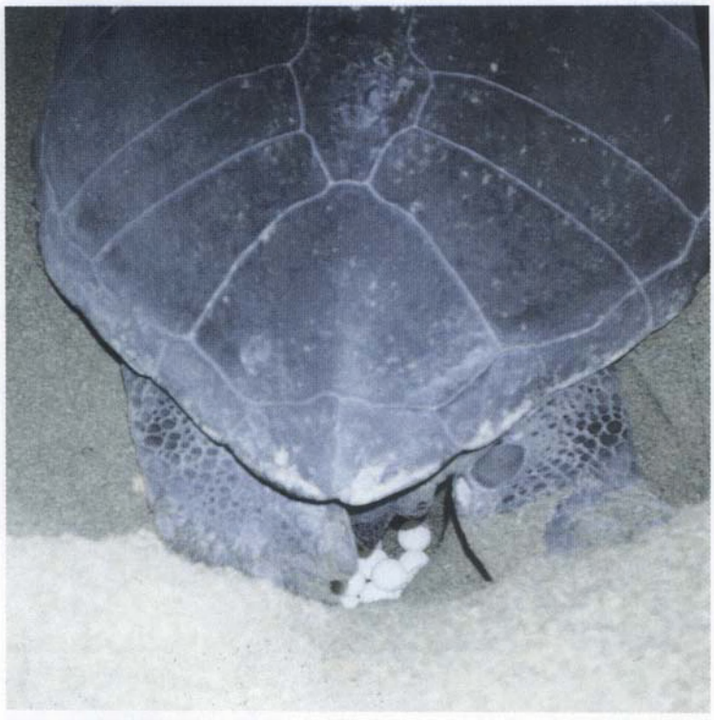

Fig. 18.28 Eggs are deposited into the chamber.

Fig. 18.29 The egg chamber is then buried. Females are often highly vocal during egg laying.

Table 18.1 Plants said to be poisonous to chelonians.

Plant	Plant part	Reference
Acokanthera	Fruit and flowers	1,2,3
Aconite (Monkshood)	Roots, flowers, leaves and seeds	1,2,3
Amaryllis	Bulb, stem, flower parts	1,2,3
Amsinckia (Tarweed)	Foliage and seeds	1,3
Anemone	Leaves, flowers	1,2,3
Apple seeds	Seeds (only if crushed!)	2,3
Apricot seeds	Inner seeds	2,3
Autumn Crocus	Bulbs	1,2,3
Avocado	Leaves	1,3
Azalea	Foliage, flowers	1,2,3
Baneberry	Foliage, fruits	1,3
Beach pea	Foliage, peas and pods	1,3
Betel-nut palm	All parts	1,3
Belladonna	Berries and other parts	1,3
Bird of Paradise	Foliage, flowers and seed pods	1,3
Bittersweet (*Solanum* spp.)	Berries	1,2,3
Black Locust	Bark, sprouts and foliage	1,3
Bleeding Heart	Foliage, flowers and roots	1,2,3
Bloodroot	All parts	1,3
Bluebonnet	Foliage and flowers	1,3
Bottlebrush	Flowers	1,2,3
Boxwood	Foliage and twigs	1,2,3
Buckeye Horse Chestnut	Sprouts and nuts	1,3
Buttercup	All parts	1,2,3
Caladium	All parts	1,2,3
Calla Lily	All parts	1,2,3
Cardinal Flower	All parts	
Carolina Jessamine	All parts	1,2,3
Cassava	Roots	1,2,3
Castor beans	Bean (uncooked 3)	1,2,3
Cherries	Twigs, seed and foliage	1,2,3
Cherry Laurel (*Prunus* spp.)	Foliage and flowers	1,2,3
Christmas Berry	Berries	3
Christmas Berry Tree	Berries	3
Christmas Cactus (*Euphorbia* spp.)	Entire plant	1,3
Christmas Rose (*Helleborus* spp.)	Foliage and flowers	1,2,3
Columbine	Foliage and flowers	1,2,3
Common Privet	Berries and leaves	1,2,3
Coral plant	All parts	1,3
Crocus	All bulbs	1,2,3
Croton	Outdoor plants, foliage, shoots	1,2,3
Cyclamen	Foliage, stems and flowers	1,3
Daffodil	Bulbs, foliage, flowers and pods	1,2,3
Daphne	Berries	1,2,3
Deadly Nightshade	Foliage, unripe fruit, sprouts	1,2,3
Death Camus	All parts, especially roots	1,3
Delphinium	Bulbs, foliage, flowers and roots	1,2,3
Destroying Angel (Death Cap)	All parts	1,3
Dogwood	Fruits mildly poisonous	2,3
Dumb Cane (*Dieffenbachia*)	Foliage	3

Table 18.1 (*cont'd*)

Plant	Plant part	Reference
Eggplant	Foliage	3
Elderberry	Leaves, shoots, bark	1,2,3
Elephant ears	Foliage	1,2,3
English Ivy	Especially berries	1,3
Eucalyptus	Fresh foliage, fruits	3
Euphorbia sp. (Poinsettia)	Foliage, flowers, latex-like sap	1,2,3
False Hellebore	All parts	1,3
Fiddleneck (Senecio)	All parts	1,3
Fly Agaric (Deathcap)	All parts	1,3
Four O'clock	All parts	1,3
Foxglove	Foliage and flowers	1,2,3
Gelsemium	All parts	1,2,3
Golden Chain	Seeds and pods	1,3
Hellebore		2
Hemlock	Roots (all parts)	1,2,3
Henbane	All parts	1,3
Holly	Leaves and berries	1,2,3
Horse Chestnut	All parts	1,2,3
Hyacinth	Bulbs, foliage and flowers	1,2,3
Hydrangea	All parts	1,2,3
Impatiens	All parts	1,2,3
Iris	Bulbs, shoots, foliage, flowers	1,2,3
Ivy	Foliage and fruit	1,2,3
Jack in the Pulpit	Roots	1,3
Jasmine	Foliage, flowers, nectar	1,2,3
Jasmine Star	Foliage, flowers	3
Jatropha	Seeds and oily sap	1,3
Jerusalem Cherry	Foliage, fruits	1,2,3
Jessamine	Berries	1,3
Jimson Weed (Thorn Apple)	Foliage, flowers and pods	1,3
Johnson Grass	(Wilted) All parts	1,3
Lambkill (Sheep Laurel)	Foliage	1,3
Lantana camara (Red Sage)	Flowers, foliage, especially berries	1,2,3
Larkspur	Entire young plant, seeds and pods	1,2,3
Laurel	All parts	1,2,3
Lily of the Valley	Foliage and flowers	1,2,3
Lobelia	All parts	1,2,3
Locoweed	All parts	1,3
Locust	All parts	1,3
Lupin	Seeds, pods and foliage	1,2,3
Marijuana	All parts	2,3
May Apple	Fruit	1,2,3
Mescal	All parts	1,3
Machineel	All parts	3
Milkweed	Foliage	1,2,3
Mistletoe	Foliage and berries	1,2,3
Moccasin Flower (Lady Slipper)	Foliage and flowers	1,2,3

Table 18.1 (*cont'd*)

Plant	Plant part	Reference
Mock Orange		2
Monkshood	Entire plant including roots	1,2,3
Moonseed	Berries	1,2,3
Morning Glory	Foliage flowers and seeds	1,2,3
Mountain Laurel	Young leaves and shoots	1,3
Mushrooms and Toadstools (Wild)	Cap and stems	1,2,3
Narcissus	Bulbs, Flowers	1,2,3
Natal Cherry (*Solanum* spp.)	Berries, foliage	1,2,3
Nectarine	Inner seed only	1,3
Nicotiana spp.	Leaves, flowers	1,2,3
Nightshades	All parts especially unripe fruits	3
Oaks	Foliage and acorns	1,2,3
Oleander	Foliage stems and flowers	1,2,3
Peach Seeds	Inner seeds	2,3
Pear Seeds	Seeds (only if crushed)	1,2,3
Peony	Foliage and flowers	1,2,3
Pennyroyal	Foliage and flowers	1,3
Periwinkle	All parts	1,2,3
Philodendron	All parts (seeds and sap)	1,2,3
Pinks	All parts	1,2,3
Plum Seeds	Inner seeds (foliage can be toxic)	1,2,3
Poinsettia	Foliage, flowers, latex-like sap	1,2,3
Poison Hemlock	Foliage and seeds	1,2,3
Poison Ivy	Foliage and fruit	1,2,3
Poison Oak	Foliage and fruit	1,2,3
Poison Sumac	Foliage and fruit	1,3
Pokeweed/pokeberry	Roots, fruit	1
Poppy	(Except Californian 1,3)	1,2,3
Potato	Sprouts, eyes and foliage	1,2,3
Privet	Berries	1,2,3
Ranunculus spp. (Buttercup)	All parts	1,2,3
Redwood	Resins when wood is wet	1,2,3
Rhododendron	Foliage and flowers	1,2,3
Rhubarb	Leaves and stems	1,2,3
Rosary Pea	Foliage, flowers and pea pods	3
Rosemary	Foliage in some species	1,2,3
Russian Thistle	Foliage and flowering parts	1,3
Sage	Foliage in some species	1,2,3
Salmonberry	Foliage and fruit	1,3
Scarlet Pimpernel	Foliage, flowers and fruit	1,3
Scotch Broom	Seeds	1,2,3
Senecio (Fiddleneck)	All parts	1,2,3
Skunk Cabbage	Roots	1,3
Snap Dragons	Foliage and flowers	1,2,3
Spanish Bayonet	Foliage and flowers	1,3
Squirrel Corn	Foliage, flowering parts and seeds	1,3
Sudan Grass	Wilted—all parts	1,3
Star of Bethlehem	Foliage and flowering parts	1,3
Sundew	Foliage	1,3
Sweet peas	Stems	1,2,3

Table 18.1 *(cont'd)*

Plant	Plant part	Reference
Tansy	Foliage and flowers	1,3
Taro spp. (Elephant's Ear)	Foliage and seeds	1,2,3
Tarweed	Flowers and seeds	1,3
Tiger Lilly	Foliage, flowers, seed pods	1,2,3
Toadflax	Foliage	1,3
Toadstools (wild types)		2
Tomato	Foliage and vines—*not* fruit	1,2,3
Toyon berry	Berry	1,3
Tree of Heaven	Foliage, flowering plants	1,3
Trillium	Foliage	1,3
Trumpet vine	All parts	1,3
Tulip	Bulbs, foliage and flowers	2,3
Venus Flytrap	Foliage, funnel, flowers	1,3
Verbena	Foliage and flowers	1,3
Vetch (several forms)	Seeds and pods	3
Virginia Creeper	Foliage, seeds and pods	1,3
Water Hemlock	Roots and foliage	1,3
Wild Black Cherry	Not specified	2
Wild parsnip	Underground roots, foliage	1,3
Wisteria	Foliage, seeds and pods	1,2,3
Yellow Star Thistle	Foliage and flowering parts	1,3
Yew	Foliage	2,3

hibernating family of herbivores. For further details the reader is referred to information detailed in earlier chapters.

RED-EARED SLIDER (TRACHEMYS SCRIPTA ELEGANS) CARE SHEET

Turtles range from Central to South America, through Mexico right to areas of Brazil. Colonies have also been noticed in Ohio, West Virginia and Kentucky. If kept under the proper conditions, red-eared turtles can live for up to 40 years, although their lifespan in captivity is usually 30 years or less. Small hatchlings are very difficult to sex with accuracy, however, mature males are often smaller than the females and develop longer claws on their front legs in comparison to that of a female. The tail of the male is also a lot wider and longer than that of the female. These creatures start off being the size of a ten-pence coin or silver dollar, eventually reaching eight to ten inches at adulthood.

Diet

Avoid fatty foods or large quantities of red meat as this may result in nutritional problems in later life.

An appropriate diet should be regularly varied. Suitable components include earthworms, whitebait, sprats, broad-leaf watercress, prawns in shell, snails, raw rabbit, chicken and turkey, crab sticks, cockles, spiders, dead mice and pinkies, kidney and liver. It is wise to supplement the diet with a source of vitamin A and a calcium/phosphorus balancer containing vitamin D (e.g. Arkvits®, Vetark, UK). A small amount of vegetable matter may be taken.

Husbandry requirements

Heat source

For the water, a thermostatically controlled water heater will be required. For the basking area, a guarded reflector (spot lamp) will be required. This basking lamp should be turned off at night.

Temperature

The water temperature should be in the region of 22°C–25°C (72°F–77°F). A basking area should be available with the temperature ranging from 28°C–31°C (82°F–86°F).

Ultraviolet (UV) light source

Turtles benefit from full-spectrum lighting (e.g. a UVB 5.0% light). This should be left on for 8–12 hours a day and replaced every six months unless otherwise stated by the manufacturer.

Filtration

This is important for keeping the water clean and to avoid foul smells. Without adequate filtration the water will acquire a heavy burden of potentially infective bacteria. Under-gravel filters do not work very well. Therefore a large internal filter or an external filter should be used if possible.

Land areas

Two land areas should be available: one for basking, with a radiant heat source above it; the other area should be away from the heat source, to allow the turtle to cool its body temperature.

Housing

Turtles grow very fast and can quickly outgrow a small fish tank. It is often far cheaper to go for a larger tank in the first place. For two adult turtles, a 120–150 cm (4–5 feet) tank would be sufficient. Indoor pools are also suitable for turtles.

Outdoor pools can be used in the hot summer months but the turtles should always be brought inside on chilly nights and during the colder months. It is crucial that animals are not released into the wild and that outdoor enclosures are secure and will not allow escape. Where local populations of red-eared sliders have become established in the wild across Europe, significant damage has been done to local reptile and amphibian populations.

Salmonella

Many reptiles carry the naturally-occurring bacteria *Salmonella*. *Salmonella* infection is contracted by ingestion (via the mouth). Good personal hygiene is therefore very important when keeping any animal and regular hand washing is encouraged. Young children and people at high risk of contracting disease because of impaired immune system function are not encouraged to handle or keep these animals.

Hibernation

Hibernation is optional and where undertaken should be in a damp environment, either in a natural outdoor enclosure or

indoors. Turtles maintained in cold, dry conditions desiccate and mostly die. A container filled with water up to a level corresponding to twice the carapace width may be used as a hibernaculum. The water can be changed every second week although turtles are extremely tolerant of long exposure to hypoxic conditions at low temperatures. (Aquatic turtles commonly hibernate in the substrate on the bottom of waters where oxygen availability is low.)

Temperatures during hibernation should be kept at 3°C–8°C. Fasting should be initiated by slowly lowering temperatures in the captive environment two to three weeks prior to hibernation. As a shorter photoperiod may be of importance to induce dormancy, the exposure to light should be decreased in parallel with temperatures. Turtles can be hibernated for two to three months. Requirements of different species in accordance with their natural habitat needs should be respected.

TESTUDO SPECIES CARE SHEET

Unfortunately, the most common cause of illness in all species of tortoise is incorrect husbandry. While tortoises should ideally be maintained outside in large enclosures for most of the year, with our British climate this is not possible and indoor accommodation is required for at least part of the year. Many tortoise owners, unlike other reptile enthusiasts, are not used to the idea of manipulating lighting, temperature and humidity in order to provide an optimal environment for their animal. Whilst some tortoises have survived for decades under these conditions, owners should realise that by keeping them under the influence of the British climate all year round, without any artificial heating and lighting, they are subjecting their tortoise to conditions which are very different from those which they have evolved to cope with. These conditions, especially in combination with a poor diet, will often lead to chronic disease. Often, disease has been developing over many years before it becomes apparent.

Accommodation

Outdoor accommodation

In the summer, when the weather is warm, tortoises should be kept outside in large, well-drained enclosures in sunny locations, which ideally should be planted with a variety of edible plants. Wild tortoises are found in scrubland areas where they range widely, feeding on high-fibre vegetation. A small pen on a lawn is not a suitable substitute. Tortoises are surprisingly agile and are often adept at climbing and burrowing. Males in particular may be hyperactive during the breeding season and pace the perimeter of their enclosure. For this reason, outdoor pens must be well-designed to prevent escape and to protect the animals from predators such as dogs, foxes, rats and birds. Tortoises are intolerant of damp and sheltered sleeping quarters should be provided.

Indoor accommodation

If left to the influence of the British climate, tortoises will hibernate for five or six months of the year, which is twice as long as they would hibernate in the wild. (Some tortoises, such as the Tunisian spur-thighed tortoises, do not hibernate at all in the wild.) Even in the summer, the temperature will not always be high enough in this country for tortoises to do well. For these reasons, all tortoise keepers should have available suitable indoor accommodation for use when outdoor climatic conditions are not suitable, e.g. in the early spring after emergence from hibernation, or during cold periods in the summer.

Indoor accommodation may be provided by utilising greenhouses, conservatories or polythene tunnels, or by setting up pens inside the house. Good ventilation is essential, and for this reason glass tanks and vivaria are generally unsuitable. Disinfectable open-topped pens are recommended.

The correct choice and depth of substrate will help in maintaining an appropriate microclimate. Juvenile Mediterranean species and Horsfield tortoises enjoy burrowing and should be provided with a substrate that permits this. Substrates commonly used for tortoises include alfalfa/grass pellets, bark chippings, hemp, newspaper, shredded paper, Astroturf, indoor/outdoor carpeting, reptile carpets and peat/soil mixtures. Sand, cat litter and crushed corn-cob or walnut shells are not recommended due to the risk of ingestion and intestinal blockage. Food should be provided on tiles or in dishes to reduce the chance of ingestion of substrate with food items.

All tortoises are best kept in small groups of one species and no new animals should be introduced without a lengthy period of quarantine. With the high incidence of viral disease in tortoises, even individuals isolated or quarantined for several years cannot be guaranteed to be free from infectious agents. Overcrowding should be avoided and animals of different sizes kept apart.

Heating

Like all reptiles, tortoises must be given the opportunity to regulate their body temperature by providing a gradient of temperatures within their appropriate temperature range (ATR) (20°C–32°C). The preferred body temperature (PBT) of a tortoise is near the upper end of this gradient (26°C–30°C) and in order for its digestion and other bodily processes to function efficiently every tortoise must be able to attain this temperature by basking in a radiant heat source during the day. Both infrared ceramic heaters and ordinary spotlights are suitable for this purpose. 40–100 W bulbs can be used depending on the height above the tortoise and the size of the enclosure. Allowing a tortoise to wander round the house will not allow it to reach the correct body temperature. Eating behaviour and activity increase dramatically when a tortoise is maintained above 26°C. The same tortoise at 21°C may move around but fail to feed properly. At night, temperatures can be allowed to fall but should not go below 18°C. As well as a basking lamp, other types of heating may be needed to keep the ambient room temperature high enough. Temperatures should be monitored by the use of thermometers, which record both maximum and minimum temperatures.

Diet

Wild tortoises feed on a wide range of vegetation, which is high in fibre and calcium and low in fat, protein and phosphorus. The ideal diet in captivity is one that mimics that of the wild as closely as possible. If this is not possible, as wide a variety of available substitutes as possible should be fed. Over-reliance on a small number of dietary components, (such as the tomato-lettuce-and-cucumber-only diet!) should be avoided. Tortoises will forage for themselves if provided with a suitably-planted large enclosure

containing edible weeds, flowers and grasses. Most owners, however, will find themselves having to rely on grocery greens and vegetables during at least part of the year. Beware of poisonous plants such as daffodils, potatoes, rhubarb, buttercup and yew.

Nutritional problems are an avoidable but common cause of disease in captive tortoises and can result from either deficiency or excess of various essential vitamins and minerals. Excessive amounts of protein will also cause problems. Animal protein such as meat, including cat and dog food, cheese and milk should never be fed.

Signs of nutritional disease are often seen at times of metabolic stress, for example growth in juveniles or in females producing eggs, and are more likely to develop if the tortoise is not kept under the correct environmental conditions.

A dietary supply of calcium is important for a variety of processes, including growth of the shell and skeleton, egg production and muscular function. Calcium metabolism and control is a complicated process relying on several organ systems and the interrelated actions of various hormones. Calcium is obtained from the food but how much of the calcium content is available to be used by the animal depends on the ratio of calcium to phosphate in the food, and the presence of calcium- and phosphate-binding chemicals, such as oxalates. Ingested calcium is absorbed from the digestive tract under the influence of parathyroid hormone and vitamin D_3. Tortoises make their own vitamin D_3 after exposure to ultraviolet light from the sun. In Britain, where tortoises need to be maintained inside for part of the year, it is recommended that artificial ultraviolet lighting is provided during this period (see below).

When considering the calcium content of the diet, it is not the total amount of calcium but the ratio of calcium to phosphorus that is important. It is generally recommended that herbivorous reptiles should be fed a diet with calcium:phosphorus ratio of at least 1.5–2:1. However, natural diets of wild tortoises typically contain a calcium:phosphorus ratio of at least 4:1 and it is possible that tortoises have a higher dietary calcium requirement than some other reptiles.

In comparison to natural forage, such as weeds, grocery greens are generally higher in protein and lower in fibre with, in many cases, an unsuitable and often inverse calcium:phosphorus ratio: for example, whereas dandelions have a calcium:phosphorus ratio of 3:1, iceberg lettuce has an inverse ratio of 0.8:1. This means that calcium supplementation is essential. Unfortunately, many of the supplements available do not have a high enough calcium:phosphorus ratio to balance deficiencies in the diet: for example, Reptivite® (ZooMed) has a ratio of 2:1 and Vionate® (Sherley's) of only 1.4:1. Nutrobal® (Vetark) and Arkvits® (Vetark), with ratios of 46:1 and 30:1 respectively, are more suitable. Daily supplementation may be necessary in reproductively-active females or actively-growing juveniles, as well as in those animals with nutritional disease. In other tortoises, supplements should not be necessary more than every other day, and a healthy tortoise grazing natural forage in the summer, or on a balanced and varied diet composed of grocery greens with the correct calcium:phosphorus ratio, may not need to be supplemented more than once weekly.

A sensible approach is to try to feed weeds and natural vegetation as much as possible along with a variety of grocery greens when necessary, each with a reasonable calcium:phosphorus ratio, and to use a calcium supplement such as Nutrobal® regularly. Suitable weeds, flowers and grasses include: dandelion, clover, plantains, sowthistle, rape, vetches, dock, chickweed and dead nettles, wild pansy, hibiscus, nasturtium, bramble, mulberry and roses. Grocery greens with a reasonable calcium:phosphorus ratio include: cabbage, turnip and beet tops, mustard greens, parsley, broccoli, Brussel's sprouts, carrot tops, romaine lettuce (not iceberg), spring/collard greens, Swiss chard, kale, spinach, endive, watercress, chicory, mint and Chinese leaves such as pak choi.

The list is slightly complicated by the fact that oxalates found in spinach, Swiss chard, cabbage and beet greens bind calcium, reducing its absorption by the digestive tract. It is thought, however, that tortoises may have evolved an ability to deal with high levels of oxalates, and as long as these items are fed sparingly, as part of a varied diet, they are unlikely to cause problems. Vegetables including: parsnips, swedes, cauliflower, carrots, courgettes, sweet potato, turnip and marrow can be given as up to 10% of the diet. Peas and beans are not recommended due to their higher protein content. Fruit including: melon, plums, pineapple, mango, figs, grapes, tomato, apple, pear, strawberries, raspberries, cucumber, peppers, watermelon and papaya should make up no more than 5% of the diet.

Grocery greens, vegetables and fruits should all be washed and dusted with a suitable mineral and vitamin supplement, such as Nutrobal® (Vetark) as discussed above. Dietary components should be chopped or shredded and well mixed to prevent selective feeding. It is important to ensure that wild plants have not been sprayed with pesticides. Similarly it would be wise if buying grocery greens to use organic produce. Complete pelleted diets are not recommended as a major dietary constituent. All tortoises must be given regular access to fresh water for drinking and bathing.

Care of juveniles

Hatchlings and juvenile tortoises are commonly kept in vivaria and often maintained year round under conditions of optimal heating, lighting and food availability. They may also be given unsuitable high-protein diets. This does not mimic the situation in the wild, where food availability and quality may vary significantly throughout the year. In addition, young wild tortoises, like adults, will not be eating during hibernation in winter and/or aestivation during hot spells. As a consequence of this unnatural, high-quality diet, many captive juveniles achieve abnormally fast rates of growth, which may be too fast for the supply of calcium available.

Metabolic bone disease (MBD) is common in these tortoises, especially if given inadequate calcium or vitamin D provision, an inappropriate dietary calcium:phosphorus ratio or lack of exposure to ultraviolet light. It can result in soft shell, shell deformity, muscular weakness, egg retention and prolapse of organs through the cloaca. Correct nutritional and environmental management will avoid metabolic bone disease, accelerated growth and early maturity. This involves providing a high fibre, low protein diet as for adults, with a high calcium supplement such as Nutrobal® (Vetark) and may include feeding every other day rather than daily. In warm weather, *Testudo* hatchlings can be housed outdoors with appropriate shelter and protection from predators.

This allows natural grazing and gives the beneficial effects of natural sunlight and exercise.

Lighting

As mentioned above, calcium is absorbed from the digestive tract under the influence of vitamin D_3. *Testudo* tortoises can produce their own vitamin D_3 via a complex biochemical pathway involving the action of ultraviolet light on cholesterol in the skin and then further processing by the liver and kidneys.

Light can be divided into infrared, visible light and ultraviolet. Ultraviolet light is further divided into UVA, B and C. UVB, which consists of wavelengths of 280–315 nm, is the range needed for vitamin D_3 production. In the wild, sunlight provides the source of ultraviolet light. Tortoises housed indoors, however, will need artificial UVB lighting if they are to produce their own vitamin D_3. This also applies to tortoises kept in greenhouses or conservatories, as UVB does not pass through glass.

There are various full spectrum ultraviolet lights available for reptiles, however they differ in the amount and wavelength of UVB which they emit. One confusing fact is that some incandescent lamps marketed as full-spectrum reptile lights do not emit any UVB at all! They provide the full-spectrum of visible light only. Of the UVB lights available it is difficult to obtain comparative data, however the Reptisun 5.0 UVB lights® (Zoo-Med), which are fluorescent tubes, Powersuns® (Zoo-med), Active UVB® (Rainbow Rock), Ultra-Vitalux® (Osram) and the Activa® (Sylvania) reptile D_3 lamps are generally considered suitable. Mixed UVB basking lamps will require a ceramic fitting due to their significant heat emission.

Even these lights have their drawbacks, however, in that the UVB wavelengths that they emit are of relatively low intensities. They must therefore be placed in close proximity (six inches) to the tortoise. In addition, the UVB output declines rapidly after six to nine months, due to build-up of deposits inside the tube, and they therefore need to be replaced at least annually, even though they are still emitting visible light. UVB-measuring devices are available through electronics suppliers.

These full-spectrum lights also emit UVA radiation which, although not needed for Vitamin D_3 synthesis, has been reported as being beneficial behaviourally and psychologically for many reptiles. Obviously, all captive tortoises should be exposed to natural sunlight whenever the weather is warm enough.

Photoperiods (i.e. the length of time lighting is provided) must be suitable for the species concerned and for species from temperate climates would naturally vary throughout the year. An example of a photoperiod chart suited to *Testudo* spp. is included in the tortoise pack.

Hibernation

In the United Kingdom, most keepers prepare their tortoises for hibernation after the autumn equinox. Persistent temperatures below 15°C in conjunction with decreasing day length will induce a tortoise kept outside to hibernate. If this is to be avoided, artificial heating and lighting in indoor accommodation will usually need to be provided from late August onwards.

Hibernating tortoises should be starved for a period of three to four weeks before entering hibernation, which often commences at about the third week in October. This is to make sure that there is no food in the digestive tract that could rot while the tortoise is hibernating. During this pre-hibernation period they should be bathed daily to encourage fluid uptake. It is important that a hibernating tortoise has a full bladder, as it can use this as a fluid store.

Hibernation should be carried out at around 5°C. This can vary by a few degrees either way but should not drop below 2°C or go above 9°C. Fridges are very suitable and popular hibernation enclosures, providing the air is changed daily and temperature control is reliable. Insulated boxes inside a cool building can also be used, provided there is adequate monitoring of both hibernation conditions and hibernation duration.

Do not put the tortoise in hay or straw. The best substrate is damp soil. This minimizes fluid loss through respiration and mimics natural conditions best, as winter is the rainy season in their natural habitat. The larger the amount of substrate the better is the protection against sudden temperature changes, as these will be buffered by the substrate. It's always difficult for us mammals not to feel uncomfortable thinking of the tortoises sleeping in a damp, cold environment. We have to overcome this and remember that these animals are reptiles and have a completely different physiology.

The maximum daytime and minimum night-time temperatures of the hibernation chamber should be checked daily. Never allow exposure to sub-zero temperatures. Tortoises can be handled carefully and checked even in a hibernating state. Weighing them regularly is useful. Expect a 1% weight loss per month. If the tortoise has urinated and lost its fluid store, it should be woken up.

Hibernating a tortoise outside is not advisable as there is a risk of frost damage, flooding and trauma from predators such as rats. In addition, if these tortoises are left to awake naturally, they will not do so until late March or early April, leading to a hibernation period of almost half a year! In the wild most tortoises will have a long period of warm weather to prepare for a short hibernation period. In Britain, tortoises may be exposed to a short period of warm weather to prepare for a long period of hibernation! This results in an increased incidence of post-hibernation problems such as mouth rot and kidney disease. The recommended maximum length of hibernation is three months for a healthy adult tortoise. This means that most tortoises will need to be awakened at the end of January or early February and kept inside in a warm enclosure until the summer.

Upon awakening, tortoises should be checked for signs of disease, such as mouth rot, discharge from the nose or eyes or swellings on the skin. All suspect animals should be taken immediately to a veterinarian. Healthy animals should be bathed twice daily in shallow warm water, encouraging drinking and voiding of urine and faeces. They should be kept in an indoor enclosure with a basking lamp and ultraviolet light as described above. A healthy tortoise should be eating within a week of ending hibernation. Appetite, urination, activity, defecation and thirst should be carefully monitored and recorded for at least three weeks following hibernation. Tortoises not seen to have urinated or eaten within a week of hibernation require veterinary attention or improvements in environment.

All tortoises will benefit from veterinary check-ups in the spring and in late summer before they go into hibernation. It is a good idea to take along a fresh faecal sample so that the vet can check for the presence of gastrointestinal parasites such as worms.

APPENDIX D: REHABILITATION OF ASIAN CHELONIANS

(Charles Innis)

Non-sustainable exploitation of many species of Asian turtles has been well publicised in recent years. Many species that were considered common only five to ten years ago have now been listed as threatened or endangered by the International Union for the Conservation of Nature (IUCN). While habitat loss and collection for the pet trade have had some impact on these species, it is generally agreed that the most significant factor affecting populations at this time is over-consumption by Asian food markets. Recently, an IUCN Asian Turtle Workshop was held in Fort Worth, Texas to address the possibility of maintaining groups of many of these species in captivity as a short-term, partial solution to this crisis. Healthy founder animals are of major importance to the establishment of captive collections.

Most Asian turtles that become available to western researchers have been collected originally via networks of local people. These well-established networks have developed over many years and result in the movement of turtles over hundreds to thousand of miles en route to food markets. During this time, the turtles are often very crowded and deprived of food and water. Western researchers or reptile dealers may acquire specimens at various points along this trade route. Clearly, prolonged transit under poor conditions results in declining health of most specimens, such that most reach the western world in poor health.

In the past, attempts to establish many of these specimens in captivity have failed. While lack of natural history information and variable stress response of certain species may be a factor, it is becoming clear that failure to address health issues of specimens seriously may be the most common reason for failure. It is this author's opinion that most, if not all, Asian chelonians can benefit from veterinary examination and treatment as soon as possible after acquisition. Experience has shown that failure to address medical issues promptly often results in death of the specimen.

QUARANTINE

In general, newly acquired animals should be placed in quarantine for three to six months. During this time, the quarantined animals are isolated from established members of the collection while they are surveyed for the presence of contagious disease. Ideally, quarantine is carried out in a building separate from the healthy collection, or at least in a room separate from the healthy collection. All husbandry items including enclosures, water bowls, sponges, etc. from the quarantine room should be kept separate from the established collection, and waste from the quarantine room should not be disposed in proximity to the established collection. The quarantine room should be serviced *after* servicing the established collection. If new animals are to enter quarantine while animals nearing the end of quarantine are present, subsets of quarantine (including new utensils etc.) should be established.

Environment

The medical management of turtles must address environmental needs, nutritional support and treatment of specific disease states. Obtaining as much natural history information as possible about the species of interest is necessary to provide a proper environment. For some species, this information is easily obtained, while for other, more poorly-known species, this information may be unknown. In such cases, it may be necessary to provide the specimens with a range of environmental conditions to be modified based on the animals' response.

In devising captive environments, important factors include choice of enclosure, temperature, substrate, humidity, photoperiod, suitable hides and shelter and presentation of water. In general, environments and enclosures for initial medical care and quarantine should be able to confine the specimens, provide appropriate water, heat, light and humidity, and should be easy to clean and disinfect. Elaborate, naturalistic vivaria should not be used during this time, as it is impossible to appropriately monitor the specimens and eradicate contagious pathogens in such conditions.

Specimens should be housed individually during quarantine if space permits. In general, plastic, glass, acrylic or fibreglass enclosures are most useful. For terrestrial species, enclosures can simply be lined with newspaper or paper towels. For aquatic and semi-aquatic species, water may be added to the desired depth with no substrate used. In either situation, the substrate or water should be discarded and the enclosure should be washed and disinfected daily. Warm water and washing-up liquid may be used to wash the enclosure, followed by disinfection with a dilute bleach solution (20 parts water to one part bleach), quaternary ammonium compound or chlorhexidine solution. Enclosures should then be thoroughly rinsed. It is generally not recommended to rely on filtration to clean water during quarantine, as pathogens may survive within the filter medium. An exception may exist where ultraviolet sterilisers are used with filtration.

Items provided within the enclosure should be kept to a minimum and should be easy to clean and disinfect. Shallow plastic trays work best for providing food and water for tortoises and semi-aquatic species. These must be shallow enough and located prominently enough for specimens to know that food and water is present, and to access it easily. Overturned plastic containers, such as plastic flowerpots cut in half, best provide hides and shelters. Animals can hide under these as well as bask on them. All enclosure furnishings should be washed and disinfected daily.

Temperature requirements vary somewhat among species, but as a rule most Asian species do well during quarantine in temperature ranges of 27°C–29°C (80°F–84°F). Some mountain aquatic species, such as *Platysternon megacephalum*, prefer much cooler temperatures. This may also be true of some forest species, such as *Geoemyda spengleri*, which seem most comfortable at 24°C–27°C (75°–80°F). If temperature requirements are unknown, a range of temperatures should be provided. Simple incandescent lights in reflector fixtures may provide basking areas for species that bask, but many forest species will avoid bright light.

In general, most Asian species prefer high humidity. The ambient humidity of the quarantine room may be kept generally high (60%–80%) and substrates may be moistened or sprayed daily.

The role of full-spectrum lighting in chelonian husbandry is poorly investigated, but such lighting may be useful with some species. In general, a day length of 12–14 hours is appropriate. Under no circumstances should lights be left on continuously. Failure to provide darkness may lead to physiological stress that exacerbates other medical problems.

Hydration and nutritional support

The vast majority of Asian turtles entering the United States have a poor nutritional status. Possibly having been deprived of food and water for weeks to months, they are often dehydrated and depleted of fat and muscle tissue. Within the first hours to days of treatment, rehydration of the specimens is vital. In some cases, simply placing an aquatic turtle in water, or placing a terrestrial species in a shallow pan of water will lead to voluntary drinking. In more severely dehydrated patients, balanced electrolyte solutions may be given by the subcutaneous, epicoelomic or intracoelomic route. In general, most chelonians can tolerate roughly 20 ml/kg/day of fluid. In very severely ill specimens, intraosseous or intravenous fluids may be needed. The importance of rehydration in restoring circulating blood volume, electrolytes, organ function and immune response cannot be overemphasised.

In rare cases, Asian chelonians will begin feeding voluntarily within the first two to three days of acquisition. If this happens, nutritional recovery is made much easier. In general, initial food offerings should be intended simply to stimulate food intake without tremendous concern over the nutritional value of the food items. For example, brightly-coloured fruits and vegetables such as strawberries, melon or yellow squash may often tempt *Indotestudo* species. Omnivores such as *Pyxidea mouhoutti* and carnivores such as *Platysternon* spp. may be tempted by earthworms. If regular feeding is established, a wider variety of items may be offered in an attempt to establish a long-term complete diet. It is unusual, however, for nutritional recovery to progress so smoothly.

Many new acquisitions will refuse to feed or may cease feeding after initially seeming enthusiastic. In the latter case, it is possible that food entering the debilitated body led to the proliferation of bacteria, fungi or parasites, or placed metabolic stresses on poorly-functioning liver and kidney tissue. As a result, nutritional support must be provided, and must often be combined with other medical therapy as discussed below. It is possible to produce the condition known as re-feeding syndrome if too much nutrition is provided too rapidly. In this situation, the body that has been chronically deprived of nutrition becomes metabolically deranged when calories are suddenly provided. To prevent this, it is best first to work on rehydrating the animal and then on gradually increasing its food intake over the first week of rehabilitation.

Nutritional support for chelonians is generally provided by tube feeding. In most cases this is accomplished by passing a feeding tube via the mouth down the oesophagus to the stomach. This technique can be performed repeatedly and safely but requires training and patience to master. In some very large specimens, tube feeding may be so difficult that placement of an oesophagostomy tube is more practical. These surgically-placed feeding tubes can be left in place for months and allow for delivery of food and medications. For most Asian species, daily tube feeding is recommended until consistent voluntary feeding is achieved.

The volume of food that can be fed at any one time varies, but, as a generality, animals can handle about 10 ml/kg at each feeding. It is important to choose a tube-feeding product that will not clog the tube and that is appropriate for the species. For herbivores, pureed vegetables, vegetable baby food or vegetable-based health-food supplements may be used. For carnivores, enteral supplements suitable for humans, dogs or cats may be used, as well as pureed dog or cat food or meat baby foods. For omnivores, a mix of these products should be used. If the patient easily tolerates once daily feeding, attempt to feed two or three times daily. Advanced techniques for nutritional support such as parenteral nutrition are being investigated.

Medical management

After establishing a plan for environmental conditions, rehydration and nutritional support, an attempt should be made to diagnose and treat specific medical problems. There are two ways to approach this phase of treatment. The first, which is often used when large numbers of common species of animals are to be rehabilitated simultaneously, is to use pre-existing knowledge of the common medical problems of the species to make assumptions about what treatments will be needed, and then apply these treatments to the entire group. The second, which is often used when small numbers of rare individuals are involved, is to use various diagnostic tests to define an individual's medical condition such that treatment may be provided in a more specific manner.

Each of these approaches to treatment has benefits and limitations. The group-treatment approach has the benefit of being less expensive and more time-efficient, as the animals can be treated in an assembly-line fashion. Its major limitation is that not all animals within the group will need all of the medications provided, and some may need medications that are not provided. Furthermore, when a good database of common disease problems is lacking for the species, as is the case with most Asian species, it is difficult to make correct assumptions about treatment. The more individualised approach has the benefit of tailoring a specific treatment to a specific disorder, but has the drawback of the expense that may be needed to define the problem and the time needed to provide different treatments to different animals. It is probably best that some aspects of both approaches are combined for specific situations.

Diagnostic investigation

A variety of diagnostic tests exists to help diagnose specific problems in chelonians. A veterinarian that is familiar with the species of interest should perform a thorough physical examination of the animal. In addition to the obvious external features, a thorough oral examination and coelomic palpation should be performed. Faecal testing to identify intestinal parasites should also be performed routinely. Such testing may involve faecal floatation, faecal cytology, faecal wet mounts, special staining techniques or assays such as immunofluorescent antibody tests. Such tests often reveal the presence of parasites such as nematodes, flagellated protozoans and amoebae. Addressing these parasites is of great importance in successful rehabilitation.

Blood biochemistry analysis and cell counts may also be useful diagnostic tests in some cases. Unfortunately, these tests lack sensitivity and it is very possible to have normal results in a very ill specimen. Radiography may be useful in diagnosing some conditions such as pneumonia, retained eggs, bladder stones and bone lesions, but is limited in its usefulness in diagnosing other serious abnormalities such as liver or kidney pathology.

Techniques for isolation or detection of specific microorganisms are very useful. These may include bacterial and fungal

cultures, PCR testing, and antibody testing for chelonian pathogens such as *Mycoplasma* and herpesvirus. Newer diagnostic tests, such as ultrasound, MRI, CT scan and endoscopy, may be useful, although availability may be limited and expense may be prohibitive. Of these, endoscopy offers tremendous value as it allows, for the first time, direct visualisation and tissue biopsy through relatively non-invasive means. Endoscopy may allow the early diagnosis of specific abnormalities and allow more accurate prognoses to be provided.

Bacterial infection

Most Asian chelonians arriving in the United States are suffering from a variety of bacterial and parasitic infections. Some may also have viral or fungal infections. Based on necropsy results of many Asian chelonians, bacterial infections are extremely common and often are the cause of death. Infections most commonly damage the digestive system, liver, kidneys and lungs. It appears that because of long transit times, dehydration and malnutrition, the turtles become immunocompromised and are susceptible to colonisation by normal enteric flora. As is true in most reptiles, Gram-negative bacteria, such as *Pseudomonas* spp., *Klebsiella* spp., *E. coli*, etc., are most commonly isolated. However, Gram-positive bacteria, such as *Streptococcus*, and anaerobic bacteria, such as *Clostridium* spp., may also be involved.

It is reasonable to assume that in almost all cases, antibiotics are of benefit in Asian turtle rehabilitation. Where specific pathogens can be isolated, antibiotic choice is based on sensitivity testing. Where cultures are not done, it is reasonable to choose a drug or combination of drugs to cover Gram-negative, Gram-positive and anaerobic organisms. The drugs most commonly used by the author (CI) are cefotaxime, chloramphenicol, trimethoprim/sulphonamide, piperacillin, enrofloxacin, amikacin and metronidazole. The length of treatment generally varies from three to six weeks depending on the severity of infection. Care should be taken to monitor for opportunistic fungal infections and maldigestion as a result of elimination of normal intestinal bacteria. It is unclear whether probiotic agents may help to prevent this problem.

Parasitism

Internal parasites are common in Asian chelonians. Parasiticides are almost always needed in Asian turtle rehabilitation. Common medications that may be used include fenbendazole for nematodes and metronidazole for some protozoans. Amoebae may be a major pathogen in some cases and may be difficult to eradicate completely. Combinations of drugs are often needed to treat amoebiasis and may include metronidazole, iodoquinol, chloroquine, diloxanide and paromomycin. Trematodes have been found in tissues of several Asian species and may respond to treatment with praziquantel. Long-term treatment (months) may be necessary to eradicate parasites. At least three negative faecal results should be obtained before releasing an animal from quarantine.

A wide range of other treatments, including antifungal drugs, nebulisation and gastroprotectants may be needed to rehabilitate Asian turtles successfully. Such treatments are still under investigation.

CONCLUSION

In many cases, Asian chelonians die despite excellent and appropriate treatment. It is critical that investigators utilise the tissues of dead specimens to increase our knowledge of the species. Failure to perform a necropsy, collect tissues for histopathology, provide tissues for chelonian genetics research and offer the cadaver to a museum collection represent a major loss of valuable information. Those working with Asian turtles on a regular basis should establish a routine for dissemination of this information to colleagues. Only by thorough tissue analysis have diseases such as intranuclear coccidiosis of tortoises been discovered. More diseases await discovery, and only by identifying these diseases will we be able to refine our treatment plans to achieve greater success.

APPENDIX E: A SELECTIVE CHELONIAN TAXONOMY

(Roger Wilkinson)

Table 18.2 includes genera of particular interest and those which might be encountered in captivity. As taxonomy is constantly being revised, the reader is referred to the websites below for up-to-date information:

- Data sourced primarily from King & Burke (1997). This is an online publication with a wealth of taxonomic and distributional data. Available at: http://www.flmnh.ufl.edu/natsci/herpetology/turtcroclist/.
- Conservation status and further information, including distribution maps, for many species can be found at the World Conservation Monitoring Centre website (UNEP WCMC 2001), http://quin.unep-mc.org/isdb/Taxonomy/tax-family-search1.cfm.

APPENDIX F: VIRAL DISEASE

(Stuart McArthur)

Tables 18.3 and 18.4 summarise some of the literature describing viral-associated disease in chelonians.

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Bacterial infection

Most Asian chelonians arriving in the United States are suffering from a variety of bacterial and parasitic infections. Some may also have viral or fungal infections. Based on necropsy results of many Asian chelonians, bacterial infections are extremely common and often are the cause of death. Infections most commonly damage the digestive system, liver, kidneys and lungs. It appears that because of long transit times, dehydration and malnutrition, the turtles become immunocompromised and are susceptible to colonisation by normal enteric flora. As is true in most reptiles, Gram-negative bacteria, such as *Pseudomonas* spp., *Klebsiella* spp., *E. coli*, etc., are most commonly isolated. However, Gram-positive bacteria, such as *Streptococcus*, and anaerobic bacteria, such as *Clostridium* spp., may also be involved.

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A wide range of other treatments, including antifungal drugs, nebulisation and gastroprotectants may be needed to rehabilitate Asian turtles successfully. Such treatments are still under investigation.

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A wide range of other treatments, including antifungal drugs, nebulisation and gastroprotectants may be needed to rehabilitate Asian turtles successfully. Such treatments are still under investigation.

CONCLUSION

In many cases, Asian chelonians die despite excellent and appropriate treatment. It is critical that investigators utilise the tissues of dead specimens to increase our knowledge of the species. Failure to perform a necropsy, collect tissues for histopathology, provide tissues for chelonian genetics research and offer the cadaver to a museum collection represent a major loss of valuable information. Those working with Asian turtles on a regular basis should establish a routine for dissemination of this information to colleagues. Only by thorough tissue analysis have diseases such as intranuclear coccidiosis of tortoises been discovered. More diseases await discovery, and only by identifying these diseases will we be able to refine our treatment plans to achieve greater success.

APPENDIX E: A SELECTIVE CHELONIAN TAXONOMY

(Roger Wilkinson)

Table 18.2 includes genera of particular interest and those which might be encountered in captivity. As taxonomy is constantly being revised, the reader is referred to the websites below for up-to-date information:

- Data sourced primarily from King & Burke (1997). This is an online publication with a wealth of taxonomic and distributional data. Available at: http://www.flmnh.ufl.edu/natsci/herpetology/turtcroclist/.
- Conservation status and further information, including distribution maps, for many species can be found at the World Conservation Monitoring Centre website (UNEP WCMC 2001), http://quin.unep-mc.org/isdb/Taxonomy/tax-family-search1.cfm.

APPENDIX F: VIRAL DISEASE

(Stuart McArthur)

Tables 18.3 and 18.4 summarise some of the literature describing viral-associated disease in chelonians.

Table 18.2 A chelonian taxonomy (Order Testudines).

Suborder	Superfamily	Family	Genus	Species	English name	Distribution & conservation status
Cryptodira	Testudinoidea	Chelydridae	*Chelydra*	*serpentina*	Snapping turtle	North and South America
			Macroclemys	*temminckii*	Alligator snapping turtle	North America, vulnerable
		Platysternidae	*Platysternidae*	*megacephalum*	Big-headed turtle	Asia, endangered
		Emydidae	*Cuora*	Various	Asian box turtles	Asia, vulnerable/endangered
			Heosemys	*grandis*	Giant Asian pond turtle	Asia, vulnerable
				spinosa	Cog-wheel turtle	Asia, endangered
			Batagur	*baska*	Giant Asian river turtle	Asia, critically endangered
			Chinemys	*reevesii*	Reeve's turtle or three-keeled pond turtle	Asia, endangered
			Cyclemys	*dentata*	Asian leaf turtle	Asia, near-threatened
			Geoemyda	*spengleri*	Leaf turtle	Asia, endangered
			Kachuga	*tecta*	Indian roofed turtle	India
			Mauremys	*leprosa, caspica, nigricans, japonica*	Mediterranean and Asiatic pond turtles	Europe, Asia, North Africa
			Melanochelys	*trijuga*	Indian black turtle or snail-eating turtle	India, Bangladesh and Indian Ocean islands, near-threatened
			Pyxidea	*mouhoutii*	Vietnamese or keeled box turtle	Asia, endangered
			Rhinoclemmys	*various*	South American wood turtles	South America, mostly near-threatened to vulnerable
			Siebenrockiella	*crassicollis*	Black marsh turtle	Asia, vulnerable
			Chrysemys	*picta*	Painted turtle	North America
			Clemmys	*guttata*	Spotted turtle	North America, vulnerable
				insculpta	Wood turtle	North America, vulnerable
				muhlenbergii	Bog turtle	North America, endangered
			Emys	*orbicularis*	European pond turtle	Europe, near-threatened
			Graptemys	*various*	Map turtles	North America, *G. flavimaculata* and *G. oculifera* endangered
			Malaclemys	*turtle*	Diamondback turtle	North America, near-threatened
			Terrapene	*ornata*	Ornate box turtle	North America, near-threatened
				carolina	Common, three-toed, Florida & Gulf Coast box turtles	North America, near-threatened

Table 18.2 (*cont'd*)

Suborder	Superfamily	Family	Genus	Species	English name	Distribution & conservation status
			Trachemys	*scripta*	Slider (numerous subspecies including red-eared, yellow-bellied and Cumberland)	North and South America, near-threatened
		Testudinae	*Furculachelys*	*whitei*	Algerian tortoise	North Africa
				nabeulensis	Tunisian tortoise	North Africa
			Geochelone	*sulcata*	African spurred tortoise	Sub-Sahara, North Africa, vulnerable
				pardalis	Leopard tortoise	Sub-Sahara, North Africa
				elegans	Indian star tortoise	India
				denticulata	Yellow-foot tortoise	South America, vulnerable
				chilensis	Chaco or Argentine tortoise	South America, vulnerable
				gigantea	Aldabran giant tortoise	Madagascar, Indian Ocean islands, Tanzania, vulnerable
				nigra/nigra	Galapagos giant tortoise	Galapagos, vulnerable
				(*Chelonoidis*)	(*Dipsochelys elephontina*)	
				carbonaria	Red-foot tortoise	South America and Caribbean
			(*Asterochelys*)	*radiata*	Radiated tortoise	Madagascar, vulnerable
			Gopherus	*agassizii*	Desert tortoise	North America, vulnerable
				berlandieri	Texas tortoise	North America
				polyphemus	Florida gopher tortoise	North America, vulnerable
				Flavomarginatus	Mexican or Bolson tortoise	North America, vulnerable
			Kinixys	*various*	African hinge-back tortoises	Africa
			Malacochersus	*tornieri*	Pancake tortoise	East Africa, vulnerable
			Psammobates	*three species*	South African tent tortoises	Southern Africa, *P. geometricus* endangered
			Testudo	*graeca*	North African or Moorish tortoise	Southern Spain and North Africa, vulnerable
				hermanni	Hermann's tortoise	Southern Europe, near-threatened
			(*Agrionemys*)	*horsfieldi*	Horsfield's, Afghan or steppe tortoise	Eurasia, vulnerable
				ibera	Greek or Turkish spur-thighed tortoise	SE Europe
				kleinmanni	Egyptian tortoise	North Africa, endangered
				marginata	Marginated tortoise	Southern Europe

Table 18.2 (*cont'd*)

Suborder	Superfamily	Family	Genus	Species	English name	Distribution & conservation status
	Trionychoidea	Dermatemydidae	Dermatemys	*mawii*	Central American river turtle	Central America, endangered
		Kinosternidae	*Kinosternon/ Sternotherus*	various	New World mud and musk turtles	North and South America
		Carretocheylidae	*Carettochelys*	*insculpta*	Australian pig-nosed turtle	Australia, vulnerable
		Trionychidae	*Apalone/ Trionyx/ Pelodiscus* and others. Nomenclature complex.	various	Soft-shelled turtles	North and South America, Asia, Africa, species too numerous to summarise status
	Chelonoidea	Cheloniidae	*Caretta*	*caretta*	Loggerhead turtle	Tropical and temperate seas, endangered
			Lepidochelys	*kempii*	Kemp's Ridley	Tropical and subtropical seas, critically endangered
				olivacea	Olive Ridley	Tropical and subtropical seas, endangered
			Chelonia	*mydas and agassizii*	Green turtles	Tropical and subtropical seas, endangered
			Eretmochelys	*imbricata*	Hawksbill turtle	Tropical and temperate seas, critically endangered
			Natator	*depressa*	Flat-backed turtle	Seas around Australia and New Guinea, vulnerable
		Dermochelyidae	*Dermochelys*	*coriacea*	Leatherback turtle	Tropical and temperate seas, critically endangered
Pleurodira		Pelomedusidae (Afro-American Sideneck turtles)	Podocnemis	*unifilis*	Yellow-spotted Amazon river turtle	South America and Caribbean, vulnerable
			Pelomedusa	*subrufa*	African helmeted turtle	Africa and Madagascar
			Pelusios	various	African sidenecked turtles	Africa
		Chelidae (Austro-American sideneck turtles)	*Chelodina*	various e.g. *longicollis*	Australian snake-necked turtles	Australia
			Chelus	*fimbriatus*	Matamata	South America
			Emydura	*subglobosa*	Red-bellied short-necked turtle	New Guinea and Australia
			Hydromedusa	*tectifera*	Argentine snake-necked turtle	South America
			Phrynops	various	South American sidenecked turtles	South America
			Platemys	various	South American chelid side-necked turtles	South America

Table 18.3 A tabulated review of early literature describing viral disease in chelonians.

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
Svet-Moldavsky *et al.* (1967)	*Testudo horsfieldi*	• Death in two out of five experimentally infected tortoises	• Sarcomas were observed in the thorax. • Tumours invaded muscles • Muscle tissue was said to have disappeared in some places.	• Sarcomas contained polymorphous, polygonal spindle-shaped and rounded cells. • The cytoplasm of polygonal cells was basophilic. • The cytoplasm of round and spindle-shaped cells was eosinophilic. • The cells contained light nuclei with chromatin granules and nucleoli.	• 4–5 year old tortoises were experimentally infected subcutaneously, intraperitoneally and intramuscularly with 30% homogenate of chicken sarcoma tissue containing the Schmidt-Ruppin retrovirus.	• It is unclear how significant these findings are, as the infection was experimentally induced. • Retroviral agents should possibly be included when considering the aetiology of unexplained infectious diseases.
Rebell *et al.* (1975) Grey-patch Disease (GPD)	*Chelonia mydas*	• Papules • Grey patches of skin • 5%–10% mortality	• Papular skin lesions • Grey skin lesion; spreading lesions 7–8 weeks after hatching	• Hyperkeratosis and acanthosis. • Basophilic intranuclear inclusions were noted by light microscopy. • Increased vascularity of dermis underlying skin lesions. • Infiltrates of mononuclear cells and eosinophilic granulocytes were present in the dermis below lesions. • In the late stages of disease, the entire epidermis is replaced by a necrotic crust of fibrin and inflammatory cells.	Electron Microscopy • Abundant viral particles were noted in scrapings and biopsies taken from spreading lesions. • Viral particles were found in the middle and upper layers of the dermis. • Particles measured 160–180 nm and contained an electron-dense core surrounded by a capsid and outer envelope. • Particles were identified as herpesvirus using morphology.	• Lesions appeared at 56–80 days after hatching. • In surviving tortoises the lesions resolved. • The authors suggest that this may be a latent virus in other marine turtles outside their study, triggered by unknown factors such as water temperature and overcrowding.
Frye *et al.* (1977) Systemic illness /death	*Clemmys marmorata*	• Lethargy • Anorexia • Muscular weakness • Death	• Petechial and ecchymotic haemorrhages beneath the skin of the limbs and neck. • Hepatomegaly. • Splenic enlargement.	• Acute hepatic necrosis • Intranuclear inclusions were observed in hepatic tissue using H&E (haematoxylin and eosin). • The stain affinity of the inclusions is not described. • Some inclusions were present in spleen, and convoluted tubules of the kidneys. • Moderate splenic hyperplasia was noted. • Haematology • Moderate lymphocytosis.	Electron Microscopy • Electron microscopy of thin sections of liver and spleen revealed intranuclear and intracytoplasmic viral particles. • The intranuclear particles measured 100 nm diameter and contained an electron-dense core. • Intracytoplasmic and extracellular particles contained an envelope and measured around 140 nm diameter.	• Disease presented in two Pacific pond turtles that had been in intimate contact with each other.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
				• Erythrocytic vacuolisation.		
Cox *et al.* (1980)	*Chrysemys picta*	• Death occurred following surgery to an aural abscess. • No viral-specific clinical signs were observed ante-mortem.	• Pulmonary oedema. • A greenish-brown and friable liver. • A distended gall bladder. • Splenic congestion. • Pale kidneys.	• Numerous randomly-scattered foci of necrosis were present throughout the liver. • A minimal inflammatory response was seen at these foci. • Hepatocytes around necrotic foci, and some more general hepatocytes, contained large eosinophilic intranuclear inclusion bodies. • Nuclei of inclusion-containing cells demonstrated margination of chromatin, with a clear halo around the inclusions. • In the lung, peribronchial accumulations of mononuclear cells and granulocytes, often with granulocytic invasion of the mucosa, were evident. • Metaplastic epithelial cells lining infundibulae contained large intranuclear eosinophilic inclusions.	Electron Microscopy • Electron microscopy of the nuclei and cytoplasm of inclusion-containing hepatocytes and bronchial epithelial cells revealed numerous viral particles. • Most particles were naked but occasional enveloped virus could be found. • Hexagonal capsids measured 85–115 nm in diameter. • Empty capsids and complete particles were seen.	• This disease is similar to that described by Frye *et al.* (1977) • Virus isolation was not attempted
Harper *et al.* (1982) Pharyngeal abscessation / necrotic stomatitis	*Gopherus agassizii*	• Death. • No clinical signs were observed prior to death.	• Poor condition with evidence of fat depletion from most reserves. • The posterior dorsal pharynx contained a 2 cm caseous necrotic plaque that occluded the glottis. • The colon was distended with food. • No other lesions were noted.	• A dense accumulation of eosinophilic, caseonecrotic debris was observed overlying a hypertrophic pharyngeal mucosa. • There was extensive vacuolation of the cytoplasm of superficial epithelial cells. • Many nuclei were shrunken and contained large homogenous eosinophilic inclusion bodies.	Electron Microscopy • Numerous enveloped icosohedral particles about 143 nm in diameter were present within cytoplasmic vesicles and free in the cytoplasm of epithelial cells. • Most particles had an electron-dense core measuring around 80 nm diameter but some empty capsids were also seen.	• A sibling in contact appeared unaffected. • Virus isolation was not attempted.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
				• Margination of chromatin was present in some affected nuclei. • A moderate infiltrate of eosinophilic granulocytes and mononuclear cells surrounded blood vessels in the submucosa. • Occasional bacterial populations were seen in the periphery of caseonecrotic material. • Mild accumulations of eosinophilic granulocytes and mononuclear cells were seen in lung sections. • Histology of other organs was unremarkable.	• Fibrous structures 100–200 nm wide and 1–3 ìm in length were noted in some nuclei along with structures with a wheel-and-spoke appearance.	
Heldstab & Bestetti (1982)	*Testudo hermanni*	• A tortoise died after 2 days of anorexia. • No clinical signs were observed prior to death.	• The liver was soft, iregularly red-yellow-grey. Multiple small spots were disseminated throughout the parenchyma. • The mucosa of the intestine was discoloured a dirty yellow. • The spleen was congested and multiple white foci were noted on the cut surface.	• Focal necrotising hepatitis. • Focal necrotising enteritis of large and small intestine with the lamina propria containing a dense infiltrate of lymphocytes and plasma cells. • Confluent necrotising splenitis. • Hepatocytes adjacent to lesions contained strongly basophilic intracytoplasmic inclusion bodies in close proximity to the nucleus. • Inclusion size varied but often reached the size of the nucleus.	Electron Microscopy • Numerous intracytoplasmic iridovirus-like particles were demonstrated using electron microscopy. • Particles were hexagonal and were located around an electron-dense nucleocapsid. • Cytoplasmic particles were enclosed by a hexagonal capsule composed of at least 2 layers. • Mean viral diameter was 140–160 µm. • A few free virions were seen in the nucleus	• Virus isolation was not attempted.
Jacobson *et al.* (1982) Systemic disease/ hepatitis/death	*Graptemys pseudogeographica; Graptemys barbouri*	• Lethargy • Anorexia • Subcutaneous oedema • Death	• Subcutaneous oedema • Hepatomegaly • Diffusely pale colouration of the liver.	• Multifocal hepatic necrosis • Diffuse hepatic necrosis • Intranuclear eosinophilic inclusions within hepatocytes	Electron Microscopy • Intranuclear and intracytoplasmic aggregation of particles morphologically resembling herpesvirus. • Intranuclear particles measured 121–159 nm.	• Attempted transmission of supernatant fluid from liver suspensions was unsuccessful. • No bacterial organisms were isolated from affected organs.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
					• Mature enveloped intracytoplasmic particles measured 175–200 nm.	
Jacobson *et al.* (1982) Cutaneous papillomas	*Platemys platycephala*	• Five turtles presented with multifocal, raised white oval skin lesions. • Ulcerative shell lesions.	• As previous column.	• Hyperkeratosis and hyperplasia of the dermis. • Cells contained pale, hypertrophic nuclei with vacuolation and marginated chromatin. • No inclusions were seen.	Electron Microscopy • Intranuclear aggregates of virus particles were observed. • The particles averaged 43 nm in diameter. • No envelopes were observed. • The particles were in crystalline arrays.	• Virus isolation was not attempted
Jacobson *et al.* (1985) Necrotic stomatitis	Geochelone *chilensis*	• Nasal discharge • Ocular discharge • Anorexia • Lethargy • Death	• Severe necrotising lesions of the oral cavity. • Serous atrophy of fat. • Pale liver.	• Mucosal epithelium had diffuse areas of necrosis with an infiltrate of mixed inflammatory cells (heterophils, lymphocytes, plasma cells and macrophages) • Large eosinophilic intranuclear inclusions were seen within many degenerating epithelial cells • Peripheral displacement of chromatin • Inclusions were not seen in other tissues beyond the oral mucosa.	Electron Microscopy • Electron microscopy confirmed the presence of viral particles considered to be herpesvirus • Particles were seen acquiring an outer envelope from the nuclear membrane. • Mature particles were seen in membrane bound cytoplasmic vesicles or were free within the cytoplasm. • Enveloped particles measured 120–130 nm	• *Geochelone carbonaria* (red-foot tortoises) that arrived with the *Geochelone chilensis* (Argentinean tortoises) remained clinically normal.
Jacobson *et al.* (1986) LETD Lung-Eye-Trachea-Disease	*Chelonia mydas*	• Gasping sounds when surfacing to breathe. • Buoyancy problems (N = 4). • Inability to dive (N = 6). • Moderate/severe lethargy (N = 5). • Caseous exudate over both eyes (N = 4).	• All turtles had moderate to severe periglottal necrosis. • Moderate amounts of caseous material were present in the trachea. • Emphysematous areas were present in one or both lungs. • Exudate was present within airways of the lungs. • Emphysema of the colon, and raised white nodules of the liver, were noted in one turtle each.	• Intranuclear inclusions were observed in periglottal and trachea epithelial cells. • Hypertrophic nuclei contained amphophilic inclusions (H&E). • Inclusion-containing cells were few in number and always located at the periphery of lesions. • Inclusion-containing epithelial and syncytial cells were seen in the major bronchi of two turtles.	Electron Microscopy • Electron microscopy confirmed the presence of viral particles considered to be herpesvirus. • Intranuclear inclusions consisted of viral particles (88–97 nm in diameter). These contained electron-dense cores. • Mature enveloped particles (132–147 nm in diameter) were seen in the cytoplasm.	• Fourteen juvenile (15–20 month old) green sea turtles were examined. These were representative of a larger group. The total number is not specified but mortality rates were 8%–38% depending upon the year.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
Cooper *et al.* (1988) Necrotic stomatitis	*Testudo graeca*	• Massive swelling of the neck. • Stomatitis affecting lower gum margins.	• Diphtheroid-membrane formation within the oral cavity.	• Oral epithelial degeneration with vasodilatation and clusters of bacteria, but little evidence of any inflammatory response.	Electron Microscopy • Electron microscopy confirmed the presence of viral particles considered to be herpesvirus or possibly adenovirus. • Large numbers of intranuclear viral particles were present in the normal bronchial and palatine epithelium. • Aggregation into paracrystalline arrays was observed. • The size of the viral particles was not stated.	• 13 tortoises from a large colony of tortoises presented with stomatitis. • In some cases the disease spread to the whole oral cavity and was terminal. • The authors suggest a favourable response to treatment using 5% acyclovir ointment (Zovirax, Wellcome).
Muller *et al.* (1988) Necrotic stomatitis and systemic disease	*Testudo hermanni*	• Inappetence • Anasarca • Rhinitis • Stomatitis • Pneumonia • Swelling of the neck • Dyspnoea	• Subcutaneous oedema. • Multiple pale foci in liver and spleen. • Hepatomegaly. • Splenomegaly. • Fibrinopurulent plaques covering the tongue. • Oedema of the brain. • Thickening of the lungs.	• Basophilic intracytoplasmic inclusion bodies were observed in spleen, liver, kidneys, trachea, digestive tract and brain. • Foci of acute necrosis with petechiation were noted within the liver. Neighbouring cells containing variable-sized basophilic intracytoplasmic inclusions. • A pyogranulomatous hepatitis consisting of heterophils and macrophages. • The epithelia of the gall bladder, bile ducts, pancreatic ducts, renal tubules, trachea, stomach and small intestine demonstrated cytoplasmic ballooning with single cell necrosis, basophilic cytoplasmic inclusions. Secondary bacteria	Electron Microscopy • The inclusions were identified as iridovirus-like using electron microscopy. • Cytoplasmic inclusions within the liver and choroid plexus demonstrated hexagonal viral particles with electron dense capsids and a layered shell. Empty envelopes were also observed. • Virions measured 135–150 nm diameter.	• Bacteria (*Aeromonas* sp.) were observed in all organs and within joints of untreated animals. • The authors suggest that latent carriers were responsible for the outbreak. • 200 out of 200 tortoises died during the outbreak. • The authors suggest many tortoises died from septicaemia.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
				and heterophils were observed. • A round cell infiltrate was noted within the small intestine. • Uniform ballooning degeneration of cells within the brain. Large areas of necrosis were noted within the ependyma and choroid plexus with small cytoplasmic inclusions.		
Braune *et al.* (1989) Necrotic stomatitis	*Testudo horsfieldi* *Testudo hermanni* *Testudo marginata* *Geochelone chilensis* *Geochelone pardalis* *Malacochersus*	• Lethargy • Inappetence • Increased secretions • Abnormal swallowing • Inability to drink • Diphtheroid necrotising stomatitis • Swelling of the neck • Occasional exfoliation of the skin of the head and neck. • Dyspnoea with open mouthed breathing and abnormal head movements. • Death was common 3–6 days after main signs.	• Diphtheroid necrotising stomatitis (thick yellow plaques overlying the oral and pharyngeal mucosa).	• Islands of intact mucosa within the upper digestive tract had large intranuclear eosinophilic inclusion bodies (Cowdry type A). • Occasional inclusion bodies were noted in adjacent connective tissue and endothelial cells. • Two tortoises had inclusion bodies in tracheal epithelium. • No other organs appeared to be affected.	Electron microscopy • Many intranuclear nucleocapsids with electron-dense cores surrounded by a shell. • Viral particles were 120–150 nm diameter. • Similar particles were observed in cytoplasm surrounded by a single or double envelope.	• Hatchlings were worst affected and signs appeared 2–4 weeks after exposure to new tortoises
Lange *et al.* (1989) Necrotic stomatitis	*Testudo hermanni*, *Testudo horsfieldi*	• Stomatitis. • 50% of 130 tortoises died over a period of 3 months. • Initially young tortoises died.	• Necrotic caseous lesions of the upper digestive tract. • Hepatomegaly and enteritis were observed in some less acute cases.	• Necrosis of the epithelium of the digestive tract with the presence of an inflammatory infiltrate. • Some animals demonstrated fungal mycelia in cells lining the upper digestive tract. • Large eosinophilic intranuclear inclusion bodies were noted in epithelial cells of the upper digestive tract. • Hepatic lipidosis.	Electron Microscopy • Concentrated and negatively-stained gut specimens revealed herpes-virus-like particles in two *T. hermanni* and one *A. horsfieldi.* • The particle size and morphology are not described. • Enveloped and naked particles 100–200 nm are illustrated. • Attempted culture in embryonic tortoise cells was unsuccessful.	• Attempted isolation in cell lines was unsuccessful.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
Muller *et al.* (1990) Necrotic stomatitis	*Testudo hermanni* *Testudo graeca*	• Stomatitis • Glossitis • Dyspnoea • Extensive parasitism	• Lesions of upper respiratory tract and liver.	• Variable degrees of tongue necrosis, from focal lesions to diphtheroid plaques. • Eosinophilic intranuclear inclusions were observed in the stratum spinosum of the tongue, pharynx, oesophagus and lower respiratory tract. • Lung tissue demonstrated bullous emphysema. • Further inclusions were observed in renal glomeruli and in the medulla oblongata and diencephalon of the brain. • Ballooning degeneration of nuclei and inclusions were also observed in spleen, liver, ductus deferens, tear glands and salivary glands.	Electron Microscopy • Hepatic and tracheal samples revealed the presence of herpes-virus-like particles. • Intranuclear nucleocapsids measuring 87–100 nm. • Mature enveloped cytoplasmic virions measuring 110–120 nm.	• 25 tortoises were examined from 13 different locations. • The authors give inclusions within epithelial cells of tongue impression smears as simple method of early diagnosis. • The authors suggest the presence of inclusions in the CNS (5/12 animals) and tear glands corresponds to glial and tear gland carriage in herpes simplex in man. • It is suggested that stress, nutrition and parasites led to recrudescence. • Vertical and horizontal transmission were suspected.
Oettle *et al.* (1990) Necrotic stomatitis, respiratory disease and high mortality	*Chersina angulata*, *Homopus areolatus*	• Anorexia • Lethargy • Purulent/ necrotic stomatitis • Occasional animals had panophthalmitis • Death • Some tortoises developed skin lesions • Hind limb paresis	• Pulmonary oedema • Necrotic enteritis • Purulent/necrotic stomatitis • Panophthalmitis	• Necrotic enteritis and the presence of fusiform bacteria. • Mycotic pneumonia. • Hepatocellular adenocarcinoma. • Renal amyloidosis. • Intranuclear inclusions (not described) in oesophageal cells. • Intracytoplasmic inclusions (not described) in oesophageal cells.	• Herpes-virus-like inclusions were noted in one animal and paramyxovirus-like inclusions in another. It is not clear if this was based upon inclusions noted by light microscopy alone.	• The colony contained four species of tortoise: *Psammobates tentoria* *Homopus areolatis* *Chersina angulata* *Geochelone pardalis* • Thirty-one tortoises died over six months following 73 tortoise introductions over 12 months, into what had previously been a colony of 10.
Jacobson *et al.* (1991) Cutaneous fibro-papillomatosis	*Chelonia mydas*	(2 Turtles) • Debility • Fibro-papillomatosis • Large cutaneous cervical tumours • Periocular tumours	Turtle 1 • An accumulation of dark coelomic fluid. • Oedema of the serosal surfaces of the digestive tract, liver, spleen, kidney and muscle fascia.	Turtle 1 • Multiple granulomas surrounding trematode eggs in liver, spleen and lung.	Electron microscopy • Intranuclear inclusions contained immature viral particles with electron-lucent and electron-dense	• It is possible that viral particles are attracted to lesions but are not their cause. • The authors suggest transmission

Table 18.3 (*cont'd*)

Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
	• Pericloacal tumours • Tumours of the cornea	• Trematode eggs were noted upon faecal examination. Turtle 2 • White foci/masses were noted in the kidneys causing distortion of shape. • White foci were observed in lung parenchyma.	• The 3 cutaneous fibropapillomas exhibited marked hyperplasia of the dermis with anastamosing rete ridges extending into the dermis. The epidermis was thrown into papillary fronds. There was marked cytoplasmic vacuolisation and ballooning degeneration of superficial cells. • Many spongiotic cells contained eosinophilic intranuclear inclusions. • The dermis underlying lesions contained proliferating fibroblasts. Turtle 2 • Most lung parenchyma was replaced by hyalinised fibrous tissue, in which trematode eggs were embedded. • Similar areas of fibromatosis were present in the kidneys, with trematode eggs scattered within and outside affected areas. • The 14 cutaneous fibropapillomas exhibited similar proliferative changes to Turtle 1. • In one small area of one of the 14 tumours there was ballooning degeneration of epidermal cells and eosinophilic intranuclear inclusions.	cores. Viral particles were also in adjacent areas of cytoplasm. • Immature particles measured 77–90 nm and obtained an envelope from the nuclear membrane. • Mature cytoplasmic particles measured from 110–120 nm. • Based upon size, location and morphogenesis both types of particles were consistent with herpesvirus.	studies may demonstrate the importance of herpesvirus in the aetiology of fibropapillomatosis. • Isolation is not discussed.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
Kabisch and Frost (1994) Necrotic stomatitis	*Testudo hermanni* *Testudo horsfieldi*	• Stomatitis • Excessive secretion of oral fluids • Soft faeces • Reduced food intake • Weight loss (up to 30% of total bodyweight in 2 days)	• Diphtheroid membrane formation in the oral cavity.	• Diptheroid/ necrosing glossitis and stomatitis. • Occasional laryngeal obstruction by necrotic material. • Intranuclear eosinophilic inclusions were present in degenerating epithelial cells of the tongue. • Similar inclusions were noted in neurons and glial cells in the brain. • Similar inclusions were noted in hepatocytes and tracheal epithelium.	Electron Microscopy • Herpesvirus particles with and without envelopes were identified. Isolation • Embryonic cells from *T. hermanni* were used in disposable tissue culture flasks to isolate the virus. • Cells were grown using Eagles minimal essential medium supplemented with 5% foetal bovine serum and antibiotics. The cultures were incubated at 26°C. • Virus was identified using electron microscopy of the third passage of the cytopathic agent. Serology • Serology of affected but surviving *T. hermanni* showed a twofold increase in neutralising antibodies. • Some *T. graeca* showed positive but stable titres. • One *T. graeca* showed a twofold rise in neutralising antibody titre.	• *T. hermanni* were affected more severely than *T. graeca.* • No *T. graeca* died but 80% of *T. hermanni* died.
Biermann (1995) Necrotic stomatitis	*Testudo hermanni*	• Ante-mortem clinical signs are not described. • All animals were dead and presented for post mortem.	Diphtheroid plaques filled the mouth and pharynx. • Sometimes the oesophagus and trachea were also affected. • One animal presented with ascites, another with a unilateral pneumonia. • Hepatic lipidosis and atrophic kidneys were also recorded.	• Intranuclear inclusion bodies were noted in the mouth pharynx and trachea of affected tortoises. • One case demonstrated intranuclear inclusions in liver tissue. • Intranuclear inclusions and a mixed cell infiltrate were observed in the gut.	Virus Isolation • Three herpes-virus isolates were obtained from three different tortoises. • After 14 days a cytopathic effect was noted in a turtle heart cell line at 28°C. • Multiple passage was possible. • All isolates were chloroform sensitive showing them to be enveloped.	• Biermann also isolated a paramyxovirus from the lung of a *Testudo hermanni* and a paramyxovirus from the lung of a snapping turtle. • The first isolation of herpesvirus in this study is described by Biermann & Blahak (1993).

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
					• Herpes-virus cultivation was reduced/ impossible at 37°C. Serology • The relationship between isolates was assessed by virus neutralisation using antibodies raised in rabbits. • 33%–87% neutralisation rates were obtained. • Biermann suggested the viruses were in 2 serogroups. • No cross neutralisation to 6 avian herpesviruses were observed.	
Westhouse *et al.* (1996)	*Gopherus polyphemus*	• Upper respiratory tract disease • Ocular and nasal mucoid discharge • Lethargy • Listlessness • Mild dehydration	• Petechial haemorrhages of the pharyngeal mucosa • Diffuse pulmonary congestion	• Ulcerated tracheal mucosa with debris containing degenerate and necrotic cells with numerous heterophils and macrophages. • Degenerate cells contained vacuoles and ovoid to round basophilic intracytoplasmic inclusions 5–9 μm in diameter. • There was diffuse fibrin exudation, heterophilic infiltration, and oedema in the lamina propria. • In the lung there was multifocal epithelial cell necrosis associated with fibrin exudation and infiltration of heterophils and macrophages. • Mild multifocal hyperplasia of cells consistent with alveolar type II cells. • No obvious bacteria or fungi observed in tissue sections of trachea and lung	Electron Microscopy • Viral particles with electron-dense spherical to icosohedral cores were observed. • Mature extracellular virions were surrounded by an envelope and were 150–220 nm in diameter. • Virions and cytoplasmic inclusions were morphologically similar to those of the family Iridovirus.	• Virus isolation was not undertaken.

Table 18.3 *(cont'd)*

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
Pettan Brewer *et al.* (1996)	*Gopherus agassizii*	• Weight loss • Anorexia • Stomatitis • Colonic impaction was suspected from radiographs	• Stomatitis • Tracheitis • Caseous material obstructing airways of lower respiratory tract. • Bronchopneumonia • Caecal and colonic impaction	• In the tongue there was severe papillary epithelial hyperplasia and desquamation of superficial layers of the epithelium with formation of a pseudomembrane. • Many oral epithelial cells contained amphophilic intranuclear inclusion bodies • Syncytial giant cells were seen with eosinophilic nuclei and marginated chromatin. • The trachea was diffusely infiltrated by large numbers of granulocytes associated with extensive necrosis of epithelium. • Cells lining the airways were hyperplastic with enlarged vacuolated nuclei. • Many cells had amphophilic intranuclear inclusions, marginated chromatin and compressed nucleoli. • Cowdry type A eosinophilic inclusions were also seen. • Pulmonary parenchyma contained multiple granulomas with Gram -ve bacterial colonies.	Electron Microscopy • Intranuclear inclusions were found to contain viral particles with round to hexagonal profiles averaging 110 nm diameter with electron-lucent or electron-dense cores. • Mature enveloped particles averaging 147 nm in diameter were seen in cytoplasm.	• Virus isolation was not undertaken.
Marschang *et al.* (1997b)	*Testudo hermanni* *(N = 10)* *Testudo graeca* *(N = 3)*	The two ill animals were both *T. hermanni* • Rhinitis • Conjunctivitis • Stomatitis • Anorexia • 13 of a group of 24 tortoises were examined	• No Chlamydia was detected from conjunctival swabs of all tortoises. • In both affected animals there was rhinitis and a stomatitis with fibrinous coating to the entire tongue. • *Yersinia* spp. and undetermined yeasts were isolated	• No inclusions were seen.	Electron Microscopy • A transmission electron micrograph of the herpesvirus isolated is included, but otherwise viral particles are not described. Isolation • Tissue and swab supernatants and	• Acyclovir and ganciclovir were demonstrated to inhibit virus replication in vitro in THC. • The authors conclude that the clinically normal *T. graeca* from which a pharyngeal swab revealed herpesvirus was

Table 18.3 *(cont'd)*

Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
		from two affected animals • No other gross post-mortem findings were noted in affected animals.		leukocytes were inoculated into turtle heart cells (THC) and chicken embryo fibroblasts (CEF). • All cultures were incubated at 28°C and observed daily. • Herpesvirus was isolated from tongue, gut, trachea, spleen, heart muscle, brain, pharynx, lung and peripheral leukocytes in *Testudo hermanni.* • Herpesvirus was isolated from the pharynx of one clinically normal *Testudo graeca.* Serology • A virus neutralisation test was used. • Tortoise blood was incubated at 56°C for 30 minutes for complement inactivation. • Serial dilutions were prepared in microtitre plates. • Herpesvirus suspension isolated from a *T. hermanni* pharyngeal swab was added to the plasma. • After 2 hours of incubation at 28°C freshly re-suspended THC were dropped into each well and the plates incubated at 28°C. • The plates were read at day 14. • No serum neutralising antibodies were detected in 9 (100%) *T. hermanni* examined. • An antibody titre of 1:32 was found in 2 (100%) *T. graeca* tested.	the source of the infection. • Isolation of herpesvirus from leucocytes of a *T. hermanni* is evidence of likely viraemia.

Table 18.3 *(cont'd)*

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
Drury *et al.* (1998)	*Geochelone pardalis* *Testudo hermanni*	• Ocular and nasal discharge • Intermittent paralysis	• Emaciation and muscle wasting • Thickening of coelomic membranes • Hepatic enlargement • Splenic enlargement	• Pancreatitis and tracheitis • Widespread lymphoproliferative changes affecting various organs	Electron Microscopy • Herpes-virus-like particles were seen in 9 out of 12 samples. Particles were seen in all six spleen samples and 3 out of 6 liver samples • The average size of the particles was 105 nm and the surface structure and icosohedral symmetry of herpesviruses were clearly visible. Virus Isolation • Virus isolation attempts were unsuccessful in a variety of cell lines including tortoise embryo fibroblasts (*Geochelone pardalis*), chick embryo fibroblasts, chick embryo liver and fat-head minnow, all at 31°C. Some thickening of the chorioallantoic membrane of specific-pathogen-free hens eggs was noted at the first passage level.	• The disease outbreak resulted in the death of 29 tortoises some of which succumbed to the disease and others of which were killed humanely on welfare grounds.
Drury *et al.* (1998)	*Testudo horsfieldi*	• Stomatitis	• Not described	• Not described	Electron Microscopy • No virions were observed in oral scrapings but a papilloma-like virus was observed in a lung-wash sample. The average particle size was 49 nm.	
Muro *et al.* (1998)	*Testudo graeca*	• Chronic rhinitis • Bilateral seromucous nasal discharge • Stomatitis • Glossitis • Dyspnoea • Eight out of 50 tortoises died. • Cutaneous ulcers were noted in two tortoises.	• Lesions were restricted to the respiratory tract and oral cavity. • Diphtheroid glossitis was found in six tortoises. • Two tortoises showed bilateral emphysema of the lungs. • Splenomegaly was	• Marked epithelial hyperplasia and the presence of a severe mixed inflammatory infiltrate was noted in oral, nasal and tracheal mucosa • Inflammatory lesions of the nasal and oral mucosae. • A catarrhal purulent bronchopneumonia.	• Intranuclear and intracytoplasmic particles, of the size, shape and distribution pattern, typical of a herpesvirus were seen. • Intranuclear and intracytoplasmic inclusions were noted in nasal,	• The chronicity of the nasal discharge is not described. • The authors suggest asymptomatic latent carriers of infection introduced the disease into the colony.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
		• Blepharitis and keratitis were seen in three tortoises.	observed in one tortoise. • Lymphocyte count, aspartate amino transferase activity, and α-globulin levels were higher in affected tortoises than similar unaffected tortoises in the same region.	• Some tortoises demonstrated eosinophilic intranuclear inclusions in epithelial cells.	lingual and tracheal epithelium. • Intranuclear virions were 130 nm diameter. • Intracytoplasmic particles were similar but showed lipid envelopes. These were 300 nm diameter.	
Marschang *et al.* (1998a)	*Testudo hermanni*	• Seven tortoises housed together that died were examined. • Presenting signs are not described.	• Hyperaemia and ulceration of the mucosa of the oral cavity, tongue, pharynx and oesophagus. • The above were covered with thick layers of yellow to orange fibrinonecrotic material	• The stratified squamous epithelium of the tongue, pharynx and oesophagus showed multifocal degeneration and necrosis of epithelial cells. • Large areas of the upper digestive tract were covered with fibrin and cellular debris. • Necrotic and infiltrative pathology was observed in large and small intestine, and cloaca. • Round or ill-defined basophilic intracytoplasmic inclusion bodies were observed in tongue, pharyngeal and oesophageal epithelial cells, and epithelial cells of the large intestine, small intestine and cloaca. • Basophilic intracytoplasmic inclusions were also noted in hepatocytes, pancreatic acinar cells and pulmonary epithelium of one animal.	Electron Microscopy • Electron microscopy of the sample isolated showed viral particles with an electron dense icosohedral core surrounded by an envelope. • Particles measured 160–190 nm Virus Isolation • The virus was able to be isolated in a wide range of cell lines at 28°C including: TH-1 (turtle heart) CEF (chicken embryo fibroblast) CELC (chicken embryo liver cells) MDBK (bovine kidney)) Vero (African green monkey kidney) PK (15) (porcine kidney) BHK-21 (hamster) SF21 (insect pupal ovarian tissue) • The virus was sensitive to IUDR and chloroform. • Virus was isolated from tongue, trachea, lung, liver, kidney, spleen, large and small intestine, cloaca and gonads.	• The authors identify this virus as differing from herpesvirus on the basis of basophilic cytoplasmic inclusions without eosinophilic intranuclear inclusions. • The cytopathic effect of round cell formation is similar to herpesvirus but it is unusual for herpesvirus to grow in multiple cell lines. • Transmission studies are considered.

Table 18.3 (*cont'd*)

	Species	Clinical signs	Gross pathology	Histopathology	Viral evidence	Comments
McArthur *et al.* (in preparation)	*Testudo hermanni* *Geochelone pardalis*	• Dyspnoea. • Serous frothy nasal and oral secretions. • Profound debility. • Many of the younger *T. hermanni* died within 12 hours of the above signs. • Corneal opacity. • Hind limb paresis. • Pale mucous membranes • Older *T. hermanni* (greater than five years) tended to be less severely affected than young (aged 1–5 years)	• Emaciation (n = 2) • Extensive muscle wasting(n = 2) • Hepatic lipidosis (n = 2) • Generalised thickening of coelomic membranes (n = 2) • Bile-duct rupture (n = 1) • Splenic congestion (n = 1) • Splenic enlargement —(solidly cellular, buff/brown) (n = 4) • Small white foci throughout liver (n = 4)	• *Geochelone pardalis* • Focal perisplenitis (n = 2) • Acute cholangiohepatitis • Pancreatitis (n = 1) • Tracheitis (n = 1) • Lymphoproliferative disease affecting liver (n = 1) *Testudo hermanni* • Widespread lymphoproliferative changes affecting primarily liver but also spleen, lungs, myocardium, epicardium, pancreas, ovaries and kidney • Lymphoplasmacytic enteritis	Electron microscopy • Herpes-virus-like particles were seen in 9 of the 12 samples submitted in the first outbreak (3 spleen, 6 liver). Their presence was demonstrated in all the splenic samples. No particles were seen in 3 of the 6 liver samples. • The average size was 105 nm in diameter and the typical surface structure and icosohedral symmetry of herpesviruses was clearly visible. • No enveloped viral particles were seen. • Samples were from five *T. hermanni* and one *G. pardalis*. • In a second outbreak, herpesvirus particles were seen in lung tissue, but not splenic or hepatic submissions.	• Virus isolation has proved elusive but is still in progress.

Table 18.4 Chelonian virus isolation studies.

	Species	Details of tortoises in study	Results
Marschang *et al.* (1997a)	*Testudo hermanni* *Testudo graeca*	• Viruses were isolated from three healthy tortoises (two *T. hermanni* and one *T. graeca*) and tracheal tissue from one *T. hermanni* with stomatitis-rhinitis complex.	Ganciclovir and acyclovir both proved effective at inhibiting *in vitro* growth of viral isolates. **DNA Cleavage patterns** • Isolation was undertaken and DNA cleavage patterns were produced for four *Testudo* herpes-virus isolates and four avian herpes-virus isolates. • There were no significant similarities in restriction enzyme patterns between avian and chelonian virus isolates. • All four chelonian isolates were similar but there were minor differences between them all.
Marschang *et al.* (1998b)	*Testudo hermanni*	• Specific pathology not described • Samples were chosen on the basis of pathological and histopathological findings suggestive of a virus infection (including inclusion bodies and unclear diagnosis)	**Irido-like virus isolation** • Irido-like viruses were isolated at 28°C in turtle heart cell lines (TH-1) and in chicken embryo fibroblasts (CEF). • Irido-like viruses were isolated from tongue, trachea, lung, liver, oesophagus, large and small intestine. • The isolates caused a round cell formation and vacuolation in both chicken embryo fibroblasts and turtle heart cells. **Lysis agent isolation** • An agent causing lysis in TH-1 cells but with no cytopathic effect in CEF was isolated from a number of organs. • The agent was not sensitive to chloroform of IUDR but could be serially passaged in TH-1. • It was able to pass through a 100 nm filter without reduction in titre. • The agent has not been visualised and appears to destroy cell cultures very quickly before other viral particles can grow.
Marschang *et al.* (1998b)	*Testudo graeca*	• Specific pathology not described • Samples were chosen on the basis of pathological and histopathological findings suggestive of a virus infection (including inclusion bodies and unclear diagnosis)	**Herpes-like virus isolation** • Herpes-like viruses were isolated at 28°C in turtle heart cell lines (TH-1) from the oesophagus of two *T. graeca* and the small intestine of one *T. graeca*. • Round-cell formation in TH-1 but no cytopathic effect in chicken embryo fibroblasts (CEF). • Histopathology also demonstrated eosinophilic inclusions in affected tissues. **Reo-like virus isolation** • A reo-like virus was isolated. • Syncytium formation in CEF, but no cytopathic effect in TH-1. • The virus was non-enveloped and possibly an RNA virus. • Lysis agent. **Lysis agent isolation** • An agent causing lysis in TH-1 cells but with no cytopathic effect in CEF was isolated from a number of organs. • The agent was not sensitive to chloroform of IUDR but could be serially passaged in TH-1. • It was able to pass through a 100 nm filter without reduction in titre. • The agent has not been visualised and appears to destroy cell cultures very quickly before other viral particles can grow.
Marschang *et al.* (1998b)	*Geochelone denticulata*	• Specific pathology not described • Samples were chosen on the basis of pathological and histopathological findings suggestive of a virus infection (including inclusion bodies and unclear diagnosis)	**Herpes-like virus isolation** • Herpes-like viruses were isolated at 28°C in turtle heart cell lines (TH-1) from the tongue, trachea and brain of one *Geochelone denticulata*. • Round-cell formation in TH-1 but no cytopathic effect in chicken embryo fibroblasts (CEF). • Histopathology also demonstrated eosinophilic inclusions in affected tissues.
Marschang *et al.* (1998b)	*Geochelone pardalis*	• Specific pathology not described • Samples were chosen on the basis of pathological and histopathological findings suggestive of a virus infection (including inclusion bodies and unclear diagnosis)	**Lysis agent isolation** • An agent causing lysis in TH-1 cells but with no cytopathic effect in CEF was isolated from a number of organs. • The agent was not sensitive to chloroform of IUDR but could be serially passaged in TH-1. • It was able to pass through a 100 nm filter without reduction in titre. • The agent has not been visualised and appears to destroy cell cultures very quickly before other viral particles can grow.

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